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DICTIONARY FILE UPDATES: 15 JAN 2006 HIGHEST RN 871978-73-3

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* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added,   *
* effective March 20, 2005.  A new display format, IDERL, is now    *
* available and contains the CA role and document type information. *
*                                                                    *
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http://www.cas.org/ONLINE/UG/regprops.html

```
                E "MD-2"/CN 7
L1            2 S E4-5
                E MD2/CN 7
L2            1 S E3
                E MD 2/CN 7
L3            3 S E3
                E MYELOID DIFFERENTIATION PROTEIN/CN
L4            6 S L1 OR L2 OR L3
```

FILE 'CAPLUS' ENTERED AT 11:04:52 ON 17 JAN 2006
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FILE COVERS 1907 - 17 Jan 2006 VOL 144 ISS 4
FILE LAST UPDATED: 16 Jan 2006 (20060116/ED)

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http://www.cas.org/infopolicy.html

L1 2 SEA FILE=REGISTRY ABB=ON PLU=ON ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR)"/CN OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246)"/CN)
L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON MD2/CN
L3 3 SEA FILE=REGISTRY ABB=ON PLU=ON "MD 2"/CN
L4 6 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2 OR L3
L5 59 SEA FILE=CAPLUS ABB=ON PLU=ON (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?)(2W)2)(L)(ENDOTOXIN OR ENDO TOXIN)
L6 25 SEA FILE=CAPLUS ABB=ON PLU=ON L5(L)(GRAM(W)(NEG OR NEGATIVE) OR MENINGITID? OR MENINGOCOCC? OR COLI OR AERUGINOSA OR INFLUENZAE OR TYPHIMURIUM OR TULARENSIS)

L6 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 14 Nov 2005
ACCESSION NUMBER: 2005:1207627 CAPLUS
DOCUMENT NUMBER: 143:458453
TITLE: Biochemical and Functional Characterization of Membrane Blebs Purified from Neisseria meningitidis Serogroup B
AUTHOR(S): Post, Deborah M. B.; Zhang, DeSheng; Eastvold, Joshua S.; Teghanemt, Athmane; Gibson, Bradford W.; Weiss, Jerrold P.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine and the Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, 52242, USA
SOURCE: Journal of Biological Chemistry (2005), 280(46), 38383-38394
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Studies with purified aggregates of **endotoxin** have revealed the importance of lipopolysaccharide-binding protein (LBP)-dependent extraction and transfer of individual **endotoxin** mols. to CD14 in Toll-like receptor 4 (TLR4)-dependent cell activation. **Endotoxin** is normally embedded in the outer membrane of intact **Gram-neg.** bacteria and shed membrane vesicles ("blebs"). However, the ability of LBP and CD14 to efficiently promote TLR4-dependent cell activation by membrane-associated **endotoxin** has not been studied extensively. In this study, the authors used an acetate auxotroph of Neisseria **meningitidis** serogroup B to facilitate metabolic labeling of bacterial **endotoxin** and compared interactions of purified **endotoxin** aggregates and of membrane-associated **endotoxin** with LBP, CD14, and **endotoxin**-responsive cells. The **endotoxin**, phospholipid, and protein composition of the recovered

blebs indicate that the blebs derive from the bacterial outer membrane. Proteomic anal. revealed an unusual enrichment in highly cationic (pI > 9) proteins. Both purified **endotoxin** aggregates and blebs activate monocytes and endothelial cells in a LBP-, CD14-, and TLR4/**MD-2**-dependent fashion, but the blebs were 3-10-fold less potent when normalized for the amount of **endotoxin** added. Differences in potency correlated with differences in efficiency of LBP-dependent delivery to and extraction of **endotoxin** by CD14. Both membrane phospholipids and **endotoxin** are extracted by LBP/soluble CD14 (sCD14) treatment, but only **endotoxin**.cntdot.sCD14 reacts with **MD-2** and activates cells. These findings indicate that the proinflammatory potency of **endotoxin** may be regulated not only by the intrinsic structural properties of **endotoxin** but also by its association with neighboring mols. in the outer membrane.

REFERENCE COUNT: 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 09 Nov 2005
ACCESSION NUMBER: 2005:1190853 CAPLUS
TITLE: Pharmacological Inhibition of Endotoxin Responses Is Achieved by Targeting the TLR4 Coreceptor, MD-2
AUTHOR(S): Visintin, Alberto; Halmen, Kristen A.; Latz, Eicke; Monks, Brian G.; Golenbock, Douglas T.
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, 01655, USA
SOURCE: Journal of Immunology (2005), 175(10), 6465-6472
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The detection of **Gram-neg.** LPS depends upon the proper function of the TLR4-**MD-2** receptor complex in immune cells. TLR4 is the signal transduction component of the LPS receptor, whereas **MD-2** is the **endotoxin** -binding unit. **MD-2** appears to activate TLR4 when bound to TLR4 and ligated by LPS. Only the monomeric form of **MD-2** was found to bind LPS and only monomeric **MD-2** interacts with TLR4. Monomeric **MD-2** binds TLR4 with an apparent Kd of 12 nM; this binding avidity was unaltered in the presence of **endotoxin**. E5564, an LPS antagonist, appears to inhibit cellular activation by competitively preventing the binding of LPS to **MD-2** . Depletion of endogenous soluble **MD-2** from human serum, with an immobilized TLR4 fusion protein, abrogated TLR4-mediated LPS responses. By determining the concentration of added-back **MD-2** that restored normal LPS responsiveness, the concentration of **MD-2** was estimated to be .apprx.50 nM. Similarly, purified TLR4-Fc fusion protein, when added to the supernatants of TLR4-expressing cells in culture, inhibited the interaction of **MD-2** with TLR4, thus preventing LPS stimulation. The ability to inhibit the effects of LPS as a result of the binding of TLR4-Fc or E5564 to **MD-2** highlights **MD-2** as the logical target for drug therapies designed to pharmacol. intervene against **endotoxin**-induced disease.

L6 ANSWER 3 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 21 Sep 2005
ACCESSION NUMBER: 2005:1016771 CAPLUS
DOCUMENT NUMBER: 143:304613
TITLE: Molecular Basis of Reduced Potency of Underacylated Endotoxins
AUTHOR(S): Teghanemt, Athmane; Zhang, DeSheng; Levis, Erika N.; Weiss, Jerrold P.; Gioannini, Theresa L.
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA, 52241, USA
SOURCE: Journal of Immunology (2005), 175(7), 4669-4676
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Potent TLR4-dependent cell activation by **Gram-neg.** bacterial **endotoxins** depends on sequential **endotoxin** -protein and protein-protein interactions with LPS-binding protein, CD14, **myeloid differentiation** protein **2** (**MD-2**), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated **endotoxins** (i.e., tetra- or penta- vs. hexa-acylated) is determined by post-CD14 interactions. To better define the mol. basis of the differences in agonist potency of **endotoxins** differing in fatty acid acylation, the authors compared **endotoxins** (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB **meningococcal** strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of **endotoxin**:protein and **endotoxin**:cell interactions, the **endotoxins** were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with **MD-2** in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS: **MD-2** and acyloxyacylhydrolase-treated LOS:**MD** -**2** were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:**MD-2** and partially antagonized the action of wt LOS:**MD-2** . These findings suggest that underacylated **endotoxins** produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin**:**MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these **endotoxin** species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin**:**MD-2** complexes.
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 06 Sep 2005
ACCESSION NUMBER: 2005:970838 CAPLUS
DOCUMENT NUMBER: 143:365556
TITLE: Activation of Toll-like receptor-mediated NF-κB by zymosan-derived water-soluble fraction: possible contribution of endotoxin-like substances

AUTHOR(S): Ikeda, Yoshihiko; Adachi, Yoshiyuki; Ishibashi, Ken-ichi; Miura, Noriko; Ohno, Naohito
CORPORATE SOURCE: Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan
SOURCE: Immunopharmacology and Immunotoxicology (2005), 27(2), 285-298
CODEN: IITOEF; ISSN: 0892-3973
PUBLISHER: Taylor & Francis, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Zymosan is a well-known reagent for the examination of inflammatory response and is prepared from yeast, Saccharomyces cerevisiae. In the activation process, Toll-like receptor (TLR) 2 and TLR6 act as functional receptors for NF-κB activation. Although zymosan is primarily composed of β-glucans, little is known about the active component of zymosan-mediated biol. activities. The active moiety of zymosan was fractionated by its solubility in water, and its biol. activity on macrophages and TLRs-transfectants examined The macrophage cell line, RAW264.7, was treated with zymosan-derived prepns., and tumor necrosis factor α (TNF-α) produced in the culture supernatant was measured by ELISA. Increased TNF-α production was observed by stimulation with water-soluble (ZWS) or water-insol. fraction (ZWIS). ZWS showed higher activity in TNF-α production NF-κB activation via TLR2, TLR1/TLR2, TLR2/TLR6, and TLR4/**MD**-**2**/CD14 also was enhanced by stimulation with ZWS and ZWIS. In particular, ZWS showed higher activity via TLR1/TLR2, TLR2/TLR6, and TLR4/**MD**-**2**/CD14 than other prepns. ZWS activity was decreased by treatment with polymyxin B, but not with lysozyme and zymolyase. Furthermore, ZWS contained more **endotoxin** than any other prepns. Apparently, the active moiety of ZWS for the NF-κB activation is an **endotoxin**-like substance, that is abundantly observed in **Gram-neg**. bacteria. These results imply that the inflammatory activity of zymosan is induced not only by β-glucans, but also by other **endotoxin**-like water-soluble substances.

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 09 Jun 2005
ACCESSION NUMBER: 2005:489362 CAPLUS
DOCUMENT NUMBER: 143:95745
TITLE: Monomeric endotoxin:protein complexes are essential for TLR4-dependent cell activation
AUTHOR(S): Gioannini, T. L.; Teghanemt, A.; Zhang, De S.; Levis, E. N.; Weiss, J. P.
CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa and the Veterans' Administration Medical Center, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2005), 11(2), 117-123
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Potent TLR4-dependent cell activation by **Gram-neg**.

bacterial **endotoxin** depends on sequential **endotoxin** -protein and protein-protein interactions with LBP, CD14, **MD** -2 and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single **endotoxin** mols. from purified **endotoxin** aggregates (Eagg) or the bacterial outer membrane and form monomeric **endotoxin**:CD14 complexes that are the preferred presentation of **endotoxin** for transfer to **MD-2**. **Endotoxin** in **endotoxin** :CD14 is readily transferred to **MD-2**, again in an albumin-dependent manner, to form monomeric **endotoxin**: **MD-2** complex. This monomeric **endotoxin** :protein complex (**endotoxin**:**MD-2**) activates TLR4 at picomolar concns., independently of albumin, and is, therefore, the apparent ligand in **endotoxin**-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of **meningococcal endotoxin** (LOS) react similarly with LBP, CD14, and **MD-2** to form **endotoxin**: **MD-2** complexes. However, tetra- and penta-acylated LOS:**MD-2** complexes are less potent TLR4 agonists than hexa-acylated LOS:**MD-2**. This is mirrored in the reduced activity of tetra-, penta- vs. hexa-acylated LOS aggregates (LOSagg) + LBP toward cells containing mCD14, **MD-2**, and TLR4. Therefore, changes in agonist potency of under-acylated **meningococcal** LOS are determined by differences in properties of monomeric **endotoxin**:**MD-2**.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 6 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 09 Jun 2005
ACCESSION NUMBER: 2005:489356 CAPLUS
DOCUMENT NUMBER: 143:72825
TITLE: Detoxifying endotoxin: Time, place and person
AUTHOR(S): Munford, Robert S.
CORPORATE SOURCE: Molecular Host Defense Laboratory, Departments of Internal Medicine and Microbiology, University of Texas Southwestern Medical School, Dallas, TX, USA
SOURCE: Journal of Endotoxin Research (2005), 11(2), 69-84
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Animals that cannot sense **endotoxin** may die if they are infected by **Gram-neg.** bacteria. Animals that sense **endotoxin** and respond too vigorously may also die, victims of their own inflammatory reactions. The outcome of **Gram-neg.** bacterial infection is thus determined not only by an individual's ability to sense **endotoxin** and respond to its presence, but also by numerous phenomena that inactivate **endotoxin** and/or prevent harmful reactions to it. **Endotoxin** sensing requires the **MD-2**/TLR4 recognition complex and occurs principally in local tissues and the liver. This review highlights the known detoxification mechanisms, which include:. (i) proteins that facilitate LPS sequestration by plasma lipoproteins, prevent interactions between the bioactive lipid A moiety and **MD-2**/TLR4, or promote cellular uptake via non-signaling pathway(s);. (ii) enzymes that deacylate or dephosphorylate lipid A;. (iii) mechanisms that remove LPS and

Gram-neg. bacteria from the bloodstream; and. (iv) neuroendocrine adaptations that modulate LPS-induced mediator production or neutralize pro-inflammatory mols. in the circulation. In general, the mechanisms for sensing and detoxifying **endotoxin** seem to be compartmentalized (local vs. systemic), dynamic, and variable between individuals. They may have evolved to confine infection and inflammation to extravascular sites of infection while preventing harmful systemic reactions. Integration of **endotoxin** sensing and detoxification is essential for successful host defense.

REFERENCE COUNT: 166 THERE ARE 166 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 19 May 2005
ACCESSION NUMBER: 2005:426231 CAPLUS
DOCUMENT NUMBER: 142:480799
TITLE: Preparation of complexes of endotoxin and MD-2 and uses thereof to modulate TLR4 receptor-dependent cell activation by endotoxin
INVENTOR(S): Weiss, Jerrold P.; Gioannini, Theresa L.; Teghanemt, Athamane; Subramanian, Ramaswamy
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 34 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005106179	A1	20050519	US 2003-715876	20031117
WO 2005049067	A1	20050602	WO 2004-US38375	20041117

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2003-715876 A 20031117

AB The disclosed invention provides purified water soluble complexes of endotoxin and MD-2. The invention also provides a method for making the complexes of the invention and a method for isolating complexes of the invention. Also provided are the method of using the complexes of the invention, e.g. method to increase or inhibit TLR4 receptor-dependent activation of cells by endotoxin in vitro or in vivo. Methods using complexes with mutant endotoxin are useful to decrease undesirable endotoxin-mediated inflammation. Methods using complexes with wild-type endotoxin are of use in promoting innate immunity and as immune adjuvants. The results of one example demonstrate that in primary cultures of human airway epithelia TLR4,

but little or no MD-2 is expressed, so the cells are relatively unresponsive to added endotoxin. However, the cell responsiveness to endotoxin is markedly amplified by either the endogenous expression or exogenous addition of MD-2.

L6 ANSWER 8 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 06 May 2005
ACCESSION NUMBER: 2005:389119 CAPLUS
DOCUMENT NUMBER: 142:480490
TITLE: Differential induction of the Toll-like receptor 4-MyD88-dependent and -independent signaling pathways by endotoxins
AUTHOR(S): Zughaier, Susu M.; Zimmer, Shanta M.; Datta, Anup; Carlson, Russell W.; Stephens, David S.
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA
SOURCE: Infection and Immunity (2005), 73(5), 2940-2950
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biol. response to **endotoxin** mediated through the Toll-like receptor 4 (TLR4)-**MD-2** receptor complex is directly related to lipid A structure or configuration. **Endotoxin** structure may also influence activation of the MyD88-dependent and -independent signaling pathways of TLR4. To address this possibility, human macrophage-like cell lines (THP-1, U937, and MM6) or murine macrophage RAW 264.7 cells were stimulated with picomolar concns. of highly purified **endotoxins**. Harvested supernatants from previously stimulated cells were also used to stimulate RAW 264.7 or 23ScCr (TLR4-deficient) macrophages (i.e., indirect induction). Neisseria **meningitidis** lipooligosaccharide (LOS) was a potent direct inducer of the MyD88-dependent pathway mols. tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 3α (MIP-3α), and the MyD88-independent mols. beta interferon (IFN-β), nitric oxide, and IFN-γ-inducible protein 10 (IP-10). Escherichia **coli** 55:B5 and Vibrio cholerae lipopolysaccharides (LPSs) at the same pmole/mL lipid A concns. induced comparable levels of TNF-α, IL-1β, and MIP-3α, but significantly less IFN-β, nitric oxide, and IP-10. In contrast, LPS from Salmonella enterica serovars Minnesota and **Typhimurium** induced amts. of IFN-β, nitric oxide, and IP-10 similar to **meningococcal** LOS but much less TNF-α and MIP-3α in time course and dose-response expts. No MyD88-dependent or -independent response to **endotoxin** was seen in TLR4-deficient cell lines (C3H/HeJ and 23ScCr) and response was restored in TLR4-**MD-2**-transfected human embryonic kidney 293 cells. Blocking the MyD88-dependent pathway by DNMyD88 resulted in significant reduction of TNF-α release but did not influence nitric oxide release. IFN-β polyclonal antibody and IFN-α/β receptor 1 antibody significantly reduced nitric oxide release. N. **meningitidis endotoxin** was a potent agonist of both the MyD88-dependent and -independent signaling pathways of the TLR4 receptor complex of human macrophages. E. **coli** 55:B5 and Vibrio cholerae LPS, at the same picomolar lipid A concns., selectively induced the MyD88-dependent pathway,

while Salmonella LPS activated the MyD88-independent pathway.
REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 22 Mar 2005
ACCESSION NUMBER: 2005:248021 CAPLUS
DOCUMENT NUMBER: 142:372121
TITLE: Crystal Structure of CD14 and Its Implications for Lipopolysaccharide Signaling
AUTHOR(S): Kim, Jung-In; Lee, Chang Jun; Jin, Mi Sun; Lee, Cherl-Ho; Paik, Sang-Gi; Lee, Hayyoung; Lee, Jie-Oh
CORPORATE SOURCE: Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon, 305-701, S. Korea
SOURCE: Journal of Biological Chemistry (2005), 280(12), 11347-11351
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Lipopolysaccharide, the **endotoxin** of **Gram-neg.** bacteria, induces extensive immune responses that can lead to fatal septic shock syndrome. The core receptors recognizing lipopolysaccharide are CD14, TLR4, and **MD-2**. CD14 binds to lipopolysaccharide and presents it to the TLR4/**MD-2** complex, which initiates intracellular signaling. In addition to lipopolysaccharide, CD14 is capable of recognizing a few other microbial and cellular products. Here, the authors present the first crystal structure of CD14 to 2.5 Å resolution A large hydrophobic pocket was found on the N-terminal side of the horseshoe-like structure. Previously identified regions involved in lipopolysaccharide binding map to the rim and bottom of the pocket indicating that the pocket is the main component of the lipopolysaccharide-binding site. Mutations that interfere with lipopolysaccharide signaling but not with lipopolysaccharide binding are also clustered in a sep. area near the pocket. Ligand diversity of CD14 could be explained by the generous size of the pocket, the considerable flexibility of the rim of the pocket, and the multiplicity of grooves available for ligand binding.
REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 07 Feb 2005
ACCESSION NUMBER: 2005:101269 CAPLUS
DOCUMENT NUMBER: 142:217303
TITLE: Oral mucosal endotoxin tolerance induction in chronic periodontitis
AUTHOR(S): Muthukuru, Manoj; Jotwani, Ravi; Cutler, Christopher W.
CORPORATE SOURCE: Department of Periodontics, School of Dental Medicine, Stony Brook University-SUNY, Stony Brook, NY, USA
SOURCE: Infection and Immunity (2005), 73(2), 687-694

CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The oral mucosa is exposed to a high d. and diversity of gram-pos. and **gram-neg**. bacteria, but very little is known about how immune homeostasis is maintained in this environment, particularly in the inflammatory disease chronic periodontitis (CP). The cells of the innate immune response recognize bacterial structures via the Toll-like receptors (TLR). This activates intracellular signaling and transcription of proteins essential for the induction of an adaptive immune response; however, if unregulated, it can lead to destructive inflammatory responses. Using single-immunoenzyme labeling, we show that the human oral mucosa (gingiva) is infiltrated by large nos. of TLR2+ and TLR4+ cells and that their nos. increase significantly in CP, relative to health ($P < 0.05$, Student's t test). We also show that the nos. of TLR2+ but not TLR4+ cells increase linearly with inflammation ($r2 = 0.33$, $P < 0.05$). Double-immunofluorescence anal. confirms that TLR2 is coexpressed by monocytes (MC)/macrophages (mϕ) in situ. Further anal. of gingival tissues by quant. real-time PCR, however, indicates that despite a threefold increase in the expression of interleukin-1β (IL-1β) mRNA during CP, there is significant (30-fold) down regulation of TLR2 mRNA ($P < 0.05$, Student's t test). Also showing similar trends are the levels of TLR4 (ninefold reduction), TLR5 (twofold reduction), and **MD-2** (sevenfold reduction) mRNA in CP patients compared to healthy persons, while the level of CD14 was unchanged. In vitro studies with human MC indicate that MC respond to an initial stimulus of lipopolysaccharide (LPS) from Porphyromonas gingivalis (PgLPS) or Escherichia **coli** (EcLPS) by upregulation of TLR2 and TLR4 mRNA and protein; moreover, IL-1β mRNA is induced and tumor necrosis factor alpha (TNF-α), IL-10, IL-6, and IL-8 proteins are secreted. However, restimulation of MC with either PgLPS or EcLPS down regulates TLR2 and TLR4 mRNA and protein and IL-1β mRNA and induces a ca. 10-fold reduction in TNF-α secretion, suggesting the induction of **endotoxin** tolerance by either LPS. Less susceptible to tolerance than TNF-α were IL-6, IL-10, and IL-8. These studies suggest that certain components of the innate oral mucosal immune response, most notably TLRs and inflammatory cytokines, may become tolerized during sustained exposure to bacterial structures such as LPS and that this may be one mechanism used in the oral mucosa to attempt to regulate local immune responses.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 29 Nov 2004
ACCESSION NUMBER: 2004:1021053 CAPLUS
DOCUMENT NUMBER: 142:73002
TITLE: Potential Role of Endotoxin as a Proinflammatory Mediator of Atherosclerosis
AUTHOR(S): Stoll, Lynn L.; Denning, Gerene M.; Weintraub, Neal L.
CORPORATE SOURCE: Department of Internal Medicine, Divisions of Cardiovascular Diseases and Infectious Diseases, University of Iowa and The VA Medical Center, Iowa City, IA, USA
SOURCE: Arteriosclerosis, Thrombosis, and Vascular Biology

(2004), 24(12), 2227-2236
CODEN: ATVBFA; ISSN: 1079-5642
PUBLISHER: Lippincott Williams & Wilkins
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Although a variety of inflammatory markers (ie, C-reactive protein) have been associated with atherosclerosis and its consequences, it is important to identify principal mediators of the inflammatory responses. One potentially important source of vascular inflammation in atherosclerosis is bacterial **endotoxin**. Mutations in Toll-like receptor 4 (TLR-4), an integral component of the **endotoxin** signaling complex, are fairly common in the Caucasian population and have recently been associated with reduced incidence of atherosclerosis and other cardiovascular diseases in some studies. Moreover, epidemiol. studies suggest that endotoxemia at levels as low as 50 pg/mL constitutes a strong risk factor for the development of atherosclerosis. **Endotoxin** concns. in this range may be produced by a variety of common subclin. **Gram-neg.** infections. In this article, we outline the main elements of the **endotoxin** signaling receptor complex that initiates proinflammatory signaling (lipopolysaccharide binding protein [LBP], CD14, TLR-4, and **MD-2**) and discuss how changes in expression of these mols. may affect proatherogenic responses in the vessel wall. We also describe some of the proinflammatory effects of **endotoxin** that may be relevant to atherosclerosis, and discuss how serum lipoproteins, especially high-d. lipoprotein, may modulate **endotoxin**-induced inflammatory responses. Further, we discuss recent findings suggesting that the lipid-lowering statins may have an addnl. protective role in blocking at least some of these proinflammatory signaling pathways. Finally, we discuss species diversity with regard to **endotoxin** signaling that should be considered when extrapolating exptl. data from animal models to humans.

REFERENCE COUNT: 175 THERE ARE 175 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 21 Sep 2004
ACCESSION NUMBER: 2004:767531 CAPLUS
DOCUMENT NUMBER: 141:393976
TITLE: Interaction of endotoxins with Toll-like receptor 4 correlates with their endotoxic potential and may explain the proinflammatory effect of Brucella spp. LPS
AUTHOR(S): Duenas, Ana I.; Orduna, Antonio; Crespo, Mariano Sanchez; Garcia-Rodriguez, Carmen
CORPORATE SOURCE: Unidad de Investigacion, Universidad de Valladolid, Department of Developmental Genetics, Hospital Clinico Universitario, Valladolid, Spain
SOURCE: International Immunology (2004), 16(10), 1467-1475
CODEN: INIMEN; ISSN: 0953-8178
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Endotoxins** displaying differences in the chemical structure of their lipid A were used to induce the expression of chemokines in the human monocytic THP-1 cell line. LPS from two enterobacterial species

such as Escherichia **coli** and Yersinia enterocolitica induced mRNA expression of IFN-γ-inducible protein (IP)-10, macrophage-inflammatory protein (MIP)-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1 and IL-8. LPS from the non-enterobacterial genera Brucella and Ochrobactrum induced the expression of these chemokines to a lower extent. Attempts to address the signaling routes involved in these responses were carried out in transiently transfected HEK293 cells. Induction of κB-driven transcriptional activity by enterobacterial LPS was observed in cells transfected with TLR-4 alone, although co-transfection of TLR-4, **MD-2** and CD14 provided optimal induction. The response to Brucella spp. and Ochrobactrum anthropi LPS was only significant at the concentration of 10 μg/mL. These data indicate that LPS from Brucella spp. and O. anthropi, which contain lipid A moieties with structural features different from those of Enterobacteriaceae elicit biochem. signaling via TLR-4 only at high concns. Neither TLR-1, TLR-2 and TLR-6 nor heterodimeric combinations of these receptor mols. are involved. Conversely, the ability of LPS to activate the TLR-4 route is a reliable mol. biomarker for endotoxicity.

REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 13 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 23 Aug 2004
ACCESSION NUMBER: 2004:686516 CAPLUS
DOCUMENT NUMBER: 141:308867
TITLE: Endotoxin responsiveness of human airway epithelia is limited by low expression of MD-2
AUTHOR(S): Jia, Hong Peng; Kline, Joel N.; Penisten, Andrea; Apicella, Michael A.; Gioannini, Theresa L.; Weiss, Jerrold; McCray, Paul B., Jr.
CORPORATE SOURCE: Department of Pediatrics, Carver College of Medicine, University of Iowa and Iowa City Veterans Administration, Iowa City, IA, USA
SOURCE: American Journal of Physiology (2004), 287(2, Pt. 1), L428-L437
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The expression of inducible antimicrobial peptides, such as human β-defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the Toll-like receptors (TLRs). We found that primary cultures of well-differentiated human airway epithelia express the mRNA for TLR-4, but little or no **MD-2** mRNA, and display little HBD-2 expression in response to treatment with purified **endotoxin** ± LPS binding protein (LBP) and soluble CD14. Expression of endogenous **MD-2** by transduction of airway epithelial cells with an adenoviral vector encoding **MD-2** or extracellular addition of recombinant **MD-2** both increased the responses of airway epithelia to **endotoxin** + LBP and sCD14 by > 100-fold, as measured by NF-κB-luciferase activity and HBD-2 mRNA expression. **MD-2** mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed

Haemophilus **influenzae**, the P6 outer membrane protein from H. **influenzae**, or TNF-α + IFN-γ). These findings suggest that **MD-2**, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to **endotoxin**. The regulation of **MD-2** expression in airway epithelia and pulmonary macrophages may serve as a means to modify **endotoxin** responsiveness in the airway.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 23 Aug 2004
ACCESSION NUMBER: 2004:683579 CAPLUS
DOCUMENT NUMBER: 141:330299
TITLE: Endotoxin recognition molecules MD-2 and toll-like receptor 4 as potential targets for therapeutic intervention of endotoxin shock
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan
SOURCE: Current Drug Targets: Inflammation & Allergy (2004), 3(3), 291-297
CODEN: CDTICU; ISSN: 1568-010X
PUBLISHER: Bentham Science Publishers Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. **Gram-neg**. sepsis is the major cause of deaths in intensive care units of hospitals and continues to increase worldwide due to the increased frequency of invasive procedures and therapy leading to immunosuppression. This syndrome is characterized by endothelial damage, coagulopathy, loss of vascular tone, tissue hypoperfusion, and multiple-organ failure. They are caused by uncontrolled, overwhelming inflammatory responses, which are triggered by microbial products. Amongst these products, **endotoxin** also called LPS (lipopolysaccharide), a constituent of the outer membrane of **Gram-neg**. bacteria, is known to play a central role by eliciting immune responses leading to production of proinflammatory cytokines. Our understanding of LPS recognition has increased dramatically over the last several years by identification of Toll-like receptor 4 (TLR4) and **MD-2** as LPS recognition mols. TLR4 is a mammalian homolog of drosophila Toll. The extracellular domain of TLR4 is associated with a mol. called **MD-2**. Mice lacking either TLR4 or **MD-2** do not respond to LPS and are resistant to **endotoxin** shock. Here, the potential for TLR4-**MD-2** as target mols. for therapeutic intervention is discussed.

REFERENCE COUNT: 88 THERE ARE 88 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 09 Apr 2004
ACCESSION NUMBER: 2004:292853 CAPLUS
DOCUMENT NUMBER: 140:401625

TITLE: Isolation of an endotoxin-MD-2 complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations

AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane; Zhang, DeSheng; Coussens, Nathan P.; Dockstader, Wendie; Ramaswamy, S.; Weiss, Jerrold P.

CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, and Department of Biochemistry Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Veterans Affairs Medical Center, Iowa City, IA, 52242, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2004), 101(12), 4186-4191
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Host proinflammatory responses to minute amts. of **endotoxins** derived from many **Gram-neg.** bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and **MD-2**. Optimal sensitivity to **endotoxin** requires an ordered series of **endotoxin**-protein and protein-protein interactions. At substoichiometric concns., LBP facilitates delivery of **endotoxin** aggregates to soluble CD14 (sCD14) to form monomeric **endotoxin**-sCD14 complexes. Subsequent interactions of **endotoxin**-sCD14 with TLR4 and/or **MD-2** have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive **endotoxin-MD-2** complex generated by treatment of **endotoxin**-sCD14 with recombinant **MD-2**. Efficient generation of this complex occurred at picomolar concns. of **endotoxin** and nanogram per mL doses of **MD-2** and required presentation of **endotoxin** to **MD-2** as a monomeric **endotoxin**-CD14 complex. TLR4-dependent delivery of **endotoxin** to human embryonic kidney (HEK) cells and cell activation at picomolar concns. of **endotoxin** occurred with the purified **endotoxin-MD-2** complex, but not with purified **endotoxin** aggregates with or without LBP and/or sCD14. The presence of excess **MD-2** inhibited delivery of **endotoxin-MD-2** to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concns. of **endotoxin** occurs by sequential interaction and transfer of **endotoxin** to LBP, CD14, and **MD-2** and simultaneous engagement of **endotoxin** and TLR4 by **MD-2**.

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN

ED Entered STN: 27 Jan 2004

ACCESSION NUMBER: 2004:64598 CAPLUS

DOCUMENT NUMBER: 140:269456

TITLE: Regulation of interactions of endotoxin with host cells

AUTHOR(S): Gioannini, Theresa L.; Teghanemt, Athmane;

Zarember, Kol A.; Weiss, Jerrold P.
CORPORATE SOURCE: Departments of Internal Medicine, Division of Infectious Diseases and The Inflammation Program, Biochemistry, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, USA
SOURCE: Journal of Endotoxin Research (2003), 9(6), 401-408
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by **endotoxin** requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of **endotoxin** to cells containing **MD-2** and TLR4. We have used metabolically labeled [14C] **meningococcal** lipooligosaccharide (LOS), purified recombinant **endotoxin**-binding proteins, and cultured endothelial cells to better define protein:**endotoxin** intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:**endotoxin** complexes or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of **endotoxin** agg (Mr≥20 + 106) to monomeric (Mr.apprx.55 + 103) **endotoxin**:sCD14 complexes. Ordered interaction of LBP (+ albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive **endotoxin** :sCD14 complex and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of **endotoxin**:sCD14 complexes and instead yielded aggregates of **endotoxin** (Mr.apprx.1-20 + 106) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of **endotoxin** selectively recognizes different protein: **endotoxin** complexes. At the outset of infection, the low concns. of LBP present and absence of extracellular BPI favor formation of pro-inflammatory **endotoxin**:CD14 complexes. The mobilization of LBP and BPI that is triggered by inflammation directs **endotoxin** for clearance and hence resolution of **endotoxin** -triggered inflammation.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 17 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 07 Jan 2004
ACCESSION NUMBER: 2004:9953 CAPLUS
DOCUMENT NUMBER: 140:92543
TITLE: Neisseria meningitidis lipooligosaccharide structure-dependent activation of the macrophage CD14/toll-like receptor 4 pathway
AUTHOR(S): Zughaier, Susu M.; Tzeng, Yih-ling; Zimmer, Shanta M.; Datta, Anup; Carlson, Russell W.; Stephens, David S.
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine,

Atlanta, GA, USA
SOURCE: Infection and Immunity (2004), 72(1), 371-380
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Meningococcal** lipopoly(oligo)saccharide (LOS) is a major inflammatory mediator of fulminant **meningococcal** sepsis and meningitis. Highly purified wild-type **meningococcal** LOS and LOS from genetically defined mutants of Neisseria **meningitidis** that contained specific mutations in LOS biosynthesis pathways were used to confirm that **meningococcal** LOS activation of macrophages was CD14/Toll-like receptor 4 (TLR4)-**MD-2** dependent and to elucidate the LOS structural requirement for TLR4 activation. Expression of TLR4 but not TLR2 was required, and antibodies to both TLR4 and CD14 blocked **meningococcal** LOS activation of macrophages. **Meningococcal** LOS α or β chain oligosaccharide structure did not influence CD14/TLR4-**MD-2** activation. However, **meningococcal** lipid A, expressed by **meningococci** with defects in 3-deoxy-D-manno-2-octulosonic acid (KDO) biosynthesis or transfer, resulted in an .apprx.10-fold reduction in biol. activity compared to KDO2-containing **meningococcal** LOS. Removal of KDO2 from LOS by acid hydrolysis also dramatically attenuated cellular responses. Competitive inhibition assays showed similar binding of glycosylated and unglycosylated lipid A to CD14/TLR4-**MD-2**. A decrease in the number of lipid A phosphate head groups or penta-acylated **meningococcal** LOS modestly attenuated biol. activity. **Meningococcal endotoxin** is a potent agonist of the macrophage CD14/TLR4-**MD-2** receptor, helping explain the fulminant presentation of **meningococcal** sepsis and meningitis. KDO2 linked to **meningococcal** lipid A was structurally required for maximal activation of the human macrophage TLR4 pathway and indicates an important role for KDO-lipid A in **endotoxin** biol. activity.
REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 18 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 13 Oct 2003
ACCESSION NUMBER: 2003:801312 CAPLUS
DOCUMENT NUMBER: 140:4026
TITLE: Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: Higher affinity than that with MD-2 or CD14
AUTHOR(S): Akashi, Sachiko; Saitoh, Shin-ichiroh; Wakabayashi, Yasutaka; Kikuchi, Takane; Takamura, Noriaki; Nagai, Yoshinori; Kusumoto, Yutaka; Fukase, Koichi; Kusumoto, Shoichi; Adachi, Yoshiyuki; Kosugi, Atsushi; Miyake, Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan
SOURCE: Journal of Experimental Medicine (2003), 198(7), 1035-1042
CODEN: JEMEAV; ISSN: 0022-1007
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Toll-like receptors (TLRs) are innate recognition mols. for microbial products, but their direct interactions with corresponding ligands remain unclarified. LPS, a membrane constituent of **gram-neg.** bacteria, is the best-studied TLR ligand and is recognized by TLR4 and **MD-2**, a mol. associated with the extracellular domain of TLR4. Although TLR4-**MD-2** recognizes LPS, little is known about the phys. interaction between LPS and TLR4-**MD-2**. Here, we demonstrate cell surface LPS-TLR4-**MD-2** complexes. CD14 greatly enhances the formation of LPS-TLR4-**MD-2** complexes, but is not copptd. with LPS-TLR4-**MD-2** complexes, suggesting a role for CD14 in LPS loading onto TLR4-**MD-2** but not in the interaction itself between LPS and TLR4-**MD-2**. A tentative dissociation constant (Kd) for LPS-TLR4-**MD-2** complexes was .apprx.3 nM, which is .apprx.10-20 times lower than the reported Kd for LPS-**MD-2** or LPS-CD14. The presence of detergent disrupts LPS interaction with CD14 but not with TLR4-**MD-2**. E5531, a lipid A antagonist developed for therapeutic intervention of **endotoxin** shock, blocks LPS interaction with TLR4-**MD-2** at a concentration 100 times lower than that required for blocking LPS interaction with CD14. These results reveal direct LPS interaction with cell surface TLR4-**MD-2** that is distinct from that with **MD-2** or CD14.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN

ED Entered STN: 23 Mar 2003

ACCESSION NUMBER: 2003:223106 CAPLUS

DOCUMENT NUMBER: 139:78507

TITLE: Inhibition of endotoxin response by E5564, a novel Toll-like receptor 4-directed endotoxin antagonist

AUTHOR(S): Mullarkey, Maureen; Rose, Jeffrey R.; Bristol, John; Kawata, Tsutomu; Kimura, Akufumi; Kobayashi, Seiichi; Przetak, Melinda; Chow, Jesse; Gusovsky, Fabian; Christ, William J.; Rossignol, Daniel P.

CORPORATE SOURCE: Biology Section, Eisai Research Institute of Boston, Inc., Andover, MA, USA

SOURCE: Journal of Pharmacology and Experimental Therapeutics (2003), 304(3), 1093-1102
CODEN: JPETAB; ISSN: 0022-3565

PUBLISHER: American Society for Pharmacology and Experimental Therapeutics

DOCUMENT TYPE: Journal

LANGUAGE: English

AB α-D-Glucopyranose,3-O-decyl-2-deoxy-6-O- [2-deoxy-3-O-[(3R)-3-methoxydecyl]-6-O-methyl-2- [[(11Z)-1-oxo-11-octade-cenyl]amino]-4-O-phosphono- β-D-glucopyranosyl]-2-[(1,3-dioxotetradecyl)amino]-1-(dihydrogen phosphate), tetrasodium salt (E5564) is a second-generation synthetic lipodisaccharide designed to antagonize the toxic effects of **endotoxin**, a major immunostimulatory component of the outer cell membrane of **Gram neg.** bacteria. In vitro, E5564 dose dependently (nanomolar concns.) inhibited lipopolysaccharide (LPS)-mediated activation of primary cultures of human myeloid cells and mouse tissue culture macrophage cell lines as well as human or animal whole blood as measured by

production of tumor necrosis factor-α and other cytokines. E5564 also blocked the ability of **Gram neg.** bacteria to stimulate human cytokine production in whole blood. In vivo, E5564 blocked induction of LPS-induced cytokines and LPS or bacterial-induced lethality in primed mice. E5564 was devoid of agonistic activity when tested both in vitro and in vivo and has no antagonistic activity against Gram pos.-mediated cellular activation at concns. up to 1 μM. E5564 blocked LPS-mediated activation of nuclear factor-κB in toll-like receptor 4/ **MD-2** -transfected cells. In a mouse macrophage cell line, activity of E5564 was independent of serum, suggesting that E5564 exerts its activity through the cell surface receptor(s) for LPS, without the need for serum LPS transfer proteins. Similar to 6-O-[2-deoxy-6-O-methyl-4-O-phosphono-3-O- (R)-3-Z-dodec-5-endoyloxydecl]-2-[3-oxo-tetradecanoylamino]-β-O-phosphono-α-D-glucopyranose tetrasodium salt (E5531), another lipid A-like antagonist, E5564 assocs. with plasma lipoproteins, causing low concns. of E5564 to be quant. inactivated in a dose- and time-dependent manner. However, compared with E5531, E5564 is a more potent inhibitor of cytokine generation, and higher doses retain activity for durations likely sufficient to permit clin. application. These results indicate that E5564 is a potent antagonist of LPS and lacks agonistic activity in human and animal model systems, making it a potentially effective therapeutic agent for treatment of disease states caused by **endotoxin.**

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 20 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 06 Feb 2003
ACCESSION NUMBER: 2003:90795 CAPLUS
DOCUMENT NUMBER: 138:203602
TITLE: Essential role of MD-2 in B-cell responses to lipopolysaccharide and Toll-like receptor 4 distribution
AUTHOR(S): Miyake, Kensuke; Nagai, Yoshinori; Akashi, Sachiko; Nagafuku, Masakazu; Ogata, Masato; Kosugi, Atsushi
CORPORATE SOURCE: Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan
SOURCE: Journal of Endotoxin Research (2002), 8(6), 449-452
CODEN: JENREB; ISSN: 0968-0519
PUBLISHER: Maney Publishing
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Toll-like receptor 4 (TLR4) mediates lipopolysaccharide (LPS) signaling in a variety of cell types. **MD-2** is associated with the extracellular domain of TLR4 and augments TLR4-dependent LPS responses in vitro. Moreover, mice lacking **MD-2** (**MD-2**-/-) do not respond to LPS, survive **endotoxin** shock, and are susceptible to Salmonella **typhimurium** infection. Here, we further show that B cells lacking **MD-2** do not up-regulate CD23 in response to LPS. TLR4 predominantly resides in the Golgi apparatus without **MD-2**. **MD-2** is essential for LPS responses in vivo.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 21 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 24 Dec 2002
ACCESSION NUMBER: 2002:972390 CAPLUS
DOCUMENT NUMBER: 138:133790
TITLE: Expression of recombinant proteins in a lipid A mutant of Escherichia coli BL21 with a strongly reduced capacity to induce dendritic cell activation and maturation
AUTHOR(S): Cognet, Isabelle; Benoit de Coignac, Amelie; Magistrelli, Giovanni; Jeannin, Pascale; Aubry, Jean-Pierre; Maisnier-Patin, Karine; Caron, Gersende; Chevalier, Sylvie; Humbert, Frederic; Nguyen, Thien; Beck, Alain; Velin, Dominique; Delneste, Yves; Malissard, Martine; Gauchat, Jean-Francois
CORPORATE SOURCE: Centre d'Immunologie Pierre-Fabre, Saint-Julien, Genevois, 74164, Fr.
SOURCE: Journal of Immunological Methods (2003), 272(1-2), 199-210
CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutations in the Escherichia **coli** (E. **coli**) and Salmonella lpxM gene have been shown to result in strains which grow normally and which produce a non-myristoylated lipopolysaccharide (nmLPS) with strongly reduced endotoxicity. Using homologous recombination, we inactivated the lpxM gene in BL21 (DE3), a strain widely used for the production of recombinant proteins. This led to a derivative unaffected in its capacity to support the production of recombinant proteins. This new strain expresses non-myristoylated LPS that induces markedly less activation and maturation of monocyte-derived dendritic cells (DC), as assessed by nuclear translocation of nuclear factor kappa B (NF-κB), production of TNF-α and IL-8 or expression of CD86. Activation of the main signal transducing receptor for extracellular LPS, Toll like receptor (TLR) 4 in conjunction with the soluble accessory protein **MD-2** was also markedly decreased. The modified BL21 strain represents a new application of lpxM inactivation for the expression of proteins to be tested on dendritic cells or other LPS sensitive cells/receptor complexes. It is likely to be useful for the identification of new proteins activating the innate immune response and to reducing the risk linked with low level of **endotoxin** contamination in therapeutic recombinant proteins.
REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 22 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 11 Jul 2002
ACCESSION NUMBER: 2002:515211 CAPLUS
DOCUMENT NUMBER: 138:37477
TITLE: Innate recognition of endotoxin from gram-negative bacteria

AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: Div. Infectious Genetics, Dep. Microbiology Immunology, Inst. Med. Sci., Univ. Tokyo, Japan
SOURCE: Saishin Igaku (2002), 57(5), 992-996
CODEN: SAIGAK; ISSN: 0370-8241
PUBLISHER: Saishin Igakusha
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review discusses the role of Toll-like receptor 4 and **MD-2** mol. in the recognition of **endotoxin** such as lipopolysaccharide from **gram-neg.** bacteria.

L6 ANSWER 23 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 15 Mar 2002
ACCESSION NUMBER: 2002:183326 CAPLUS
DOCUMENT NUMBER: 136:308499
TITLE: Response to Neisseria gonorrhoeae by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling
AUTHOR(S): Fichorova, Raina N.; Cronin, Amanda O.; Lien, Egil; Anderson, Deborah J.; Ingalls, Robin R.
CORPORATE SOURCE: Fearing Research Laboratory, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, USA
SOURCE: Journal of Immunology (2002), 168(5), 2424-2432
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Toll-like receptors (TLRs) have recently been identified as fundamental components of the innate immune response to bacterial pathogens. We investigated the role of TLR signaling in immune defense of the mucosal epithelial cells of the lower female genital tract. This site provides first line defense against microbial pathogens while remaining tolerant to a complex biosystem of resident microbiota. Epithelial cells derived from normal human vagina, ectocervix, and endocervix expressed mRNA for TLR1, -2, -3, -5, and -6. However, they failed to express TLR4 as well as **MD2**, two essential components of the receptor complex for LPS in phagocytes and endothelial cells. Consistent with this, endocervical epithelial cells were unresponsive to protein-free prepns. of lipooligosaccharide from Neisseria gonorrhoeae and LPS from Escherichia **coli**. However, they were capable of responding to whole **Gram-neg.** bacteria and bacterial lysates, as demonstrated by NF-κB activation and proinflammatory cytokine production The presence of soluble CD14, a high-affinity receptor for LPS and other bacterial ligands, enhanced the sensitivity of genital tract epithelial cells to both low and high concns. of bacteria, suggesting that soluble CD14 can act as a coreceptor for non-TLR4 ligands. These data demonstrate that the response to N. gonorrhoeae and other **Gram-neg.** bacteria at the mucosal surface of the female genital tract occurs in the absence of **endotoxin** recognition and TLR4-mediated signaling.
REFERENCE COUNT: 85 THERE ARE 85 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 24 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN

ED Entered STN: 08 Jul 2001
ACCESSION NUMBER: 2001:491171 CAPLUS
DOCUMENT NUMBER: 136:149763
TITLE: Molecular genetic analysis of an endotoxin nonresponder mutant cell line: a point mutation in a conserved region of MD-2 abolishes endotoxin-induced signaling
AUTHOR(S): Schromm, Andra B.; Lien, Egil; Henneke, Philipp; Chow, Jesse C.; Yoshimura, Atsutoshi; Heine, Holger; Latz, Eicke; Monks, Brian G.; Schwartz, David A.; Miyake, Kensuke; Golenbock, Douglas T.
CORPORATE SOURCE: Evans Biomedical Research Center, Boston University School of Medicine, Boston, MA, 02118, USA
SOURCE: Journal of Experimental Medicine (2001), 194(1), 79-88
CODEN: JEMEAV; ISSN: 0022-1007
PUBLISHER: Rockefeller University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Somatic cell mutagenesis is a powerful tool for characterizing receptor systems. We reported previously two complementation groups of mutant cell lines derived from CD14-transfected Chinese hamster ovary-K1 fibroblasts defective in responses to bacterial endotoxin. Both classes of mutants expressed a normal gene product for Toll-like receptor (TLR)4, and fully responded to stimulation by tumor necrosis factor (TNF)-α or interleukin (IL)-1β. We identified the lesion in one of the complementation groups in the gene for MD-2, a putative TLR4 coreceptor. The nonresponder phenotype of this mutant was reversed by transfection with MD-2. Cloning of MD-2 from the nonresponder cell line revealed a point mutation in a highly conserved region resulting in a C95Y amino acid exchange. Both forms of MD-2 colocalized with TLR4 on the cell surface after transfection, but only the wild-type cDNA reverted the lipopolysaccharide (LPS) nonresponder phenotype. Furthermore, soluble MD-2, but not soluble MD-2C95Y, functioned to enable LPS responses in cells that expressed TLR4. Thus, MD-2 is a required component of the LPS signaling complex and can function as a soluble receptor for cells that do not otherwise express it. We hypothesize that MD-2 conformationally affects the extracellular domain of TLR4, perhaps resulting in a change in affinity for LPS or functioning as a portion of the true ligand for TLR4.
REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 22 Mar 2001
ACCESSION NUMBER: 2001:207573 CAPLUS
DOCUMENT NUMBER: 135:316980
TITLE: LPS induction of gene expression in human monocytes
AUTHOR(S): Guha, M.; Mackman, N.
CORPORATE SOURCE: Departments of Immunology, The Scripps Research Institute, La Jolla, CA, 92037, USA
SOURCE: Cellular Signalling (2001), 13(2), 85-94
CODEN: CESIEY; ISSN: 0898-6568
PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with refs. Lipopolysaccharide [LPS (**endotoxin**)] is the principal component of the outer membrane of **Gram-neg.** bacteria. Recent studies have elucidated how LPS is recognized by monocytes and macrophages of the innate immune system. Human monocytes are exquisitely sensitive to LPS and respond by expressing many inflammatory cytokines. LPS binds to LPS-binding protein (LBP) in plasma and is delivered to the cell surface receptor CD14. Next, LPS is transferred to the transmembrane signaling receptor toll-like receptor 4 (TLR4) and its accessory protein **MD2**. LPS stimulation of human monocytes activates several intracellular signaling pathways that include the IκB kinase (IKK)-NF-κB pathway and 3 mitogen-activated protein kinase (MAPK) pathways: extracellular signal-regulated kinases (ERK) 1 and 2, c-Jun N-terminal kinase (JNK), and p38. These signaling pathways in turn activate a variety of transcription factors that include NF-κB (p50/p65) and AP-1 (c-Fos/c-Jun), which coordinate the induction of many genes encoding inflammatory mediators.
REFERENCE COUNT: 152 THERE ARE 152 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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L7 123 S L6
L8 30 DUP REM L7 (93 DUPLICATES REMOVED)

L8 ANSWER 1 OF 30 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2005-354786 [36] WPIDS
DOC. NO. CPI: C2005-109712
TITLE: New purified complexes comprising endotoxin bound to MD-2, useful for promoting innate immune response, as immunological adjuvants, or for treating conditions associated with endotoxin-mediated cell activation, e.g. sepsis.
DERWENT CLASS: B04 D16

INVENTOR(S): GIOANNINI, T L; SUBRAMANIAN, R; TEGHANEMT, A; WEISS, J P
PATENT ASSIGNEE(S): (GIOA-I) GIOANNINI T L; (SUBR-I) SUBRAMANIAN R; (TEGH-I) TEGHANEMT A; (WEIS-I) WEISS J P; (IOWA) UNIV IOWA RES FOUND
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 2005106179	A1	20050519	(200536)*		34
WO 2005049067	A1	20050602	(200536)	EN	

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IS IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 2005106179	A1	US 2003-715876	20031117
WO 2005049067	A1	WO 2004-US38375	20041117

PRIORITY APPLN. INFO: US 2003-715876 20031117
AN 2005-354786 [36] WPIDS
AB US2005106179 A UPAB: 20050608
NOVELTY - A purified complex comprising endotoxin bound to MD-2, is new.
ACTIVITY - Antibacterial; Immunosuppressive; Hepatotropic; Gastrointestinal-Gen.; Antiinflammatory; CNS-Gen.; Respiratory-Gen.; Antiasthmatic; Cytostatic.
No biological data given.
MECHANISM OF ACTION - Gene therapy; Vaccine.
USE - The complex is useful for decreasing undesirable endotoxin-mediated inflammation, or for promoting innate immune response and as immunological adjuvants. The complex and methods may be used for treating conditions associated with endotoxin-mediated cell activation, such as sepsis, liver disease, inflammatory bowel disease, cystic fibrosis, asthma, autoimmune diseases, cancer or bacterial infections.
Dwg.0/14

L8 ANSWER 2 OF 30 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005606783 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16103114
TITLE: Biochemical and functional characterization of membrane blebs purified from Neisseria meningitidis serogroup B.
AUTHOR: Post Deborah M B; Zhang DeSheng; Eastvold Joshua S; Teghanemt Athmane; Gibson Bradford W; Weiss Jerrold P
CORPORATE SOURCE: The Buck Institute for Age Research, Novato, California 94945, USA.
CONTRACT NUMBER: AI18571 (NIAID)
AI59372 (NIAID)

P0144642
SOURCE: The Journal of biological chemistry, (2005 Nov 18) 280 (46) 38383-94. Electronic Publication: 2005-08-15. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200601
ENTRY DATE: Entered STN: 20051116
Last Updated on STN: 20060111
Entered Medline: 20060110

AB Studies with purified aggregates of **endotoxin** have revealed the importance of lipopolysaccharide-binding protein (LBP)-dependent extraction and transfer of individual **endotoxin** molecules to CD14 in Toll-like receptor 4 (TLR4)-dependent cell activation. **Endotoxin** is normally embedded in the outer membrane of intact **Gram-negative** bacteria and shed membrane vesicles ("blebs"). However, the ability of LBP and CD14 to efficiently promote TLR4-dependent cell activation by membrane-associated **endotoxin** has not been studied extensively. In this study, we used an acetate auxotroph of Neisseria **meningitidis** serogroup B to facilitate metabolic labeling of bacterial **endotoxin** and compared interactions of purified **endotoxin** aggregates and of membrane-associated **endotoxin** with LBP, CD14, and **endotoxin**-responsive cells. The **endotoxin**, phospholipid, and protein composition of the recovered blebs indicate that the blebs derive from the bacterial outer membrane. Proteomic analysis revealed an unusual enrichment in highly cationic (pI > 9) proteins. Both purified **endotoxin** aggregates and blebs activate monocytes and endothelial cells in a LBP-, CD14-, and TLR4/**MD-2** -dependent fashion, but the blebs were 3-10-fold less potent when normalized for the amount of **endotoxin** added. Differences in potency correlated with differences in efficiency of LBP-dependent delivery to and extraction of **endotoxin** by CD14. Both membrane phospholipids and **endotoxin** are extracted by LBP/soluble CD14 (sCD14) treatment, but only **endotoxin**.sCD14 reacts with **MD-2** and activates cells. These findings indicate that the proinflammatory potency of **endotoxin** may be regulated not only by the intrinsic structural properties of **endotoxin** but also by its association with neighboring molecules in the outer membrane.

L8 ANSWER 3 OF 30 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2005171418 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15644310
TITLE: Crystal structure of CD14 and its implications for lipopolysaccharide signaling.
AUTHOR: Kim Jung-In; Lee Chang Jun; Jin Mi Sun; Lee Cherl-Ho; Paik Sang-Gi; Lee Hayyoung; Lee Jie-Oh
CORPORATE SOURCE: Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea.
SOURCE: Journal of biological chemistry, (2005 Mar 25) 280 (12) 11347-51. Electronic Publication: 2005-01-10. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1WWL
ENTRY MONTH: 200504
ENTRY DATE: Entered STN: 20050405
Last Updated on STN: 20050422
Entered Medline: 20050421

AB Lipopolysaccharide, the **endotoxin** of **Gram-negative** bacteria, induces extensive immune responses that can lead to fatal septic shock syndrome. The core receptors recognizing lipopolysaccharide are CD14, TLR4, and **MD-2**. CD14 binds to lipopolysaccharide and presents it to the TLR4/**MD-2** complex, which initiates intracellular signaling. In addition to lipopolysaccharide, CD14 is capable of recognizing a few other microbial and cellular products. Here, we present the first crystal structure of CD14 to 2.5 angstroms resolution. A large hydrophobic pocket was found on the NH2-terminal side of the horseshoe-like structure. Previously identified regions involved in lipopolysaccharide binding map to the rim and bottom of the pocket indicating that the pocket is the main component of the lipopolysaccharide-binding site. Mutations that interfere with lipopolysaccharide signaling but not with lipopolysaccharide binding are also clustered in a separate area near the pocket. Ligand diversity of CD14 could be explained by the generous size of the pocket, the considerable flexibility of the rim of the pocket, and the multiplicity of grooves available for ligand binding.

L8 ANSWER 4 OF 30 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2005593451 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16272300
TITLE: Pharmacological inhibition of endotoxin responses is achieved by targeting the TLR4 coreceptor, MD-2.
AUTHOR: Visintin Alberto; Halmen Kristen A; Latz Eicke; Monks Brian G; Golenbock Douglas T
CORPORATE SOURCE: Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01655, USA.. alberto.visintin@umassmws.edu
CONTRACT NUMBER: AI 52455 (NIAID)
RO1 GM54060 (NIGMS)
RR14466 (NCRR)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2005 Nov 15) 175 (10) 6465-72.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200601
ENTRY DATE: Entered STN: 20051108
Last Updated on STN: 20060104
Entered Medline: 20060103

AB The detection of **Gram-negative** LPS depends upon the proper function of the TLR4-**MD-2** receptor complex in immune cells. TLR4 is the signal transduction component of the LPS receptor, whereas **MD-2** is the **endotoxin**-binding unit. **MD-2** appears to activate TLR4 when bound to TLR4 and ligated by LPS. Only the monomeric form of **MD-2** was found to bind LPS and only monomeric **MD-2** interacts with TLR4. Monomeric **MD-2** binds TLR4 with an apparent Kd of

12 nM; this binding avidity was unaltered in the presence of **endotoxin**. E5564, an LPS antagonist, appears to inhibit cellular activation by competitively preventing the binding of LPS to **MD-2**. Depletion of endogenous soluble **MD-2** from human serum, with an immobilized TLR4 fusion protein, abrogated TLR4-mediated LPS responses. By determining the concentration of added-back **MD-2** that restored normal LPS responsiveness, the concentration of **MD-2** was estimated to be approximately 50 nM. Similarly, purified TLR4-Fc fusion protein, when added to the supernatants of TLR4-expressing cells in culture, inhibited the interaction of **MD-2** with TLR4, thus preventing LPS stimulation. The ability to inhibit the effects of LPS as a result of the binding of TLR4-Fc or E5564 to **MD-2** highlights **MD-2** as the logical target for drug therapies designed to pharmacologically intervene against **endotoxin**-induced disease.

L8 ANSWER 5 OF 30 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2005505043 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16177114
TITLE: Molecular basis of reduced potency of underacylated endotoxins.
AUTHOR: Teghanemt Athmane; Zhang DeSheng; Levis Erika N; Weiss Jerrold P; Gioannini Theresa L
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Coralville, IA 52241, USA.
CONTRACT NUMBER: AI59372 (NIAID)
PO144642
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2005 Oct 1) 175 (7) 4669-76.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200511
ENTRY DATE: Entered STN: 20050923
Last Updated on STN: 20051215
Entered Medline: 20051128

AB Potent TLR4-dependent cell activation by **gram-negative** bacterial **endotoxins** depends on sequential **endotoxin**-protein and protein-protein interactions with LPS-binding protein, CD14, **myeloid differentiation** protein **2** (**MD-2**), and TLR4. Previous studies have suggested that reduced agonist potency of underacylated **endotoxins** (i.e., tetra- or penta- vs hexa-acylated) is determined by post-CD14 interactions. To better define the molecular basis of the differences in agonist potency of **endotoxins** differing in fatty acid acylation, we compared **endotoxins** (lipooligosaccharides (LOS)) from hexa-acylated wild-type (wt), penta-acylated mutant msbB **meningococcal** strains as well as tetra-acylated LOS generated by treatment of wt LOS with the deacylating enzyme, acyloxyacylhydrolase. To facilitate assay of **endotoxin**:protein and **endotoxin**:cell interactions, the **endotoxins** were purified after metabolic labeling with [3H]- or [14C]acetate. All LOS species tested formed monomeric complexes with **MD-2** in an LPS-binding protein- and CD14-dependent manner with similar efficiency. However, msbB LOS: **MD-2** and acyloxyacylhydrolase-treated LOS:**MD**

-2 were at least 10-fold less potent in inducing TLR4-dependent cell activation than wt LOS:**MD-2** and partially antagonized the action of wt LOS:**MD-2** . These findings suggest that underacylated **endotoxins** produce decreased TLR4-dependent cell activation by altering the interaction of the **endotoxin:MD-2** complex with TLR4 in a way that reduces receptor activation. Differences in potency among these **endotoxin** species is determined not by different aggregate properties, but by different properties of monomeric **endotoxin:MD-2** complexes.

L8 ANSWER 6 OF 30 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2005211589 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15845500
TITLE: Differential induction of the toll-like receptor 4-MyD88-dependent and -independent signaling pathways by endotoxins.
AUTHOR: Zughaier Susu M; Zimmer Shanta M; Datta Anup; Carlson Russell W; Stephens David S
CORPORATE SOURCE: Division of Infectious Diseases, Emory University School of Medicine, VAMC (I-151), 1670 Clairmont Rd, Atlanta, GA 30033, USA.. szughai@emory.edu
CONTRACT NUMBER: R01 AI033517-10 (NIAID)
SOURCE: Infection and immunity, (2005 May) 73 (5) 2940-50. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 20050423
Last Updated on STN: 20050608
Entered Medline: 20050607

AB The biological response to **endotoxin** mediated through the Toll-like receptor 4 (TLR4)-**MD-2** receptor complex is directly related to lipid A structure or configuration. **Endotoxin** structure may also influence activation of the MyD88-dependent and -independent signaling pathways of TLR4. To address this possibility, human macrophage-like cell lines (THP-1, U937, and MM6) or murine macrophage RAW 264.7 cells were stimulated with picomolar concentrations of highly purified **endotoxins**. Harvested supernatants from previously stimulated cells were also used to stimulate RAW 264.7 or 23ScCr (TLR4-deficient) macrophages (i.e., indirect induction). Neisseria **meningitidis** lipooligosaccharide (LOS) was a potent direct inducer of the MyD88-dependent pathway molecules tumor necrosis factor alpha (TNF-alpha), interleukin-1beta (IL-1beta), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 3alpha (MIP-3alpha), and the MyD88-independent molecules beta interferon (IFN-beta), nitric oxide, and IFN-gamma-inducible protein 10 (IP-10). Escherichia **coli** 55:B5 and Vibrio cholerae lipopolysaccharides (LPSs) at the same pmole/ml lipid A concentrations induced comparable levels of TNF-alpha, IL-1beta, and MIP-3alpha, but significantly less IFN-beta, nitric oxide, and IP-10. In contrast, LPS from Salmonella enterica serovars Minnesota and **Typhimurium** induced amounts of IFN-beta, nitric oxide, and IP-10 similar to **meningococcal** LOS but much less TNF-alpha and MIP-3alpha in time course and dose-response experiments. No

MyD88-dependent or -independent response to **endotoxin** was seen in TLR4-deficient cell lines (C3H/HeJ and 23ScCr) and response was restored in TLR4-**MD-2**-transfected human embryonic kidney 293 cells. Blocking the MyD88-dependent pathway by DNMyD88 resulted in significant reduction of TNF-alpha release but did not influence nitric oxide release. IFN-beta polyclonal antibody and IFN-alpha/beta receptor 1 antibody significantly reduced nitric oxide release. N. **meningitidis endotoxin** was a potent agonist of both the MyD88-dependent and -independent signaling pathways of the TLR4 receptor complex of human macrophages. E. **coli** 55:B5 and Vibrio cholerae LPS, at the same picomolar lipid A concentrations, selectively induced the MyD88-dependent pathway, while Salmonella LPS activated the MyD88-independent pathway.

L8 ANSWER 7 OF 30 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2005037362 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15664906
TITLE: Oral mucosal endotoxin tolerance induction in chronic periodontitis.
AUTHOR: Muthukuru Manoj; Jotwani Ravi; Cutler Christopher W
CORPORATE SOURCE: Department of Periodontics, School of Dental Medicine, 110 Rockland Hall, Stony Brook University-SUNY, Stony Brook, NY 11794-8703, USA.
CONTRACT NUMBER: R01 DE 14328 (NIDCR)
SOURCE: Infection and immunity, (2005 Feb) 73 (2) 687-94. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200503
ENTRY DATE: Entered STN: 20050125
Last Updated on STN: 20050312
Entered Medline: 20050311

AB The oral mucosa is exposed to a high density and diversity of gram-positive and **gram-negative** bacteria, but very little is known about how immune homeostasis is maintained in this environment, particularly in the inflammatory disease chronic periodontitis (CP). The cells of the innate immune response recognize bacterial structures via the Toll-like receptors (TLR). This activates intracellular signaling and transcription of proteins essential for the induction of an adaptive immune response; however, if unregulated, it can lead to destructive inflammatory responses. Using single-immunoenzyme labeling, we show that the human oral mucosa (gingiva) is infiltrated by large numbers of TLR2(+) and TLR4(+) cells and that their numbers increase significantly in CP, relative to health ($P < 0.05$, Student's t test). We also show that the numbers of TLR2(+) but not TLR4(+) cells increase linearly with inflammation ($r(2) = 0.33$, $P < 0.05$). Double-immunofluorescence analysis confirms that TLR2 is coexpressed by monocytes (MC)/macrophages (mphi) in situ. Further analysis of gingival tissues by quantitative real-time PCR, however, indicates that despite a threefold increase in the expression of interleukin-1beta (IL-1beta) mRNA during CP, there is significant (30-fold) downregulation of TLR2 mRNA ($P < 0.05$, Student's t test). Also showing similar trends are the levels of TLR4 (ninefold reduction), TLR5 (twofold reduction), and **MD-2** (sevenfold reduction) mRNA in CP patients compared to healthy persons, while the level of CD14 was unchanged. In vitro studies with human MC indicate that MC respond to an initial stimulus of lipopolysaccharide

(LPS) from Porphyromonas gingivalis (PgLPS) or Escherichia **coli** (EcLPS) by upregulation of TLR2 and TLR4 mRNA and protein; moreover, IL-1beta mRNA is induced and tumor necrosis factor alpha (TNF-alpha), IL-10, IL-6, and IL-8 proteins are secreted. However, restimulation of MC with either PgLPS or EcLPS downregulates TLR2 and TLR4 mRNA and protein and IL-1beta mRNA and induces a ca. 10-fold reduction in TNF-alpha secretion, suggesting the induction of **endotoxin** tolerance by either LPS. Less susceptible to tolerance than TNF-alpha were IL-6, IL-10, and IL-8. These studies suggest that certain components of the innate oral mucosal immune response, most notably TLRs and inflammatory cytokines, may become tolerized during sustained exposure to bacterial structures such as LPS and that this may be one mechanism used in the oral mucosa to attempt to regulate local immune responses.

L8 ANSWER 8 OF 30 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2005447855 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 16114511
TITLE: Activation of toll-like receptor-mediated NF-kappa beta by zymosan-derived water-soluble fraction: possible contribution of endotoxin-like substances.
AUTHOR: Ikeda Yoshihiko; Adachi Yoshiyuki; Ishibashi Ken-ichi; Miura Noriko; Ohno Naohito
CORPORATE SOURCE: Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.
SOURCE: Immunopharmacology and immunotoxicology, (2005) 27 (2) 285-98.
Journal code: 8800150. ISSN: 0892-3973.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20050824
Last Updated on STN: 20051215

AB Zymosan is a well-known reagent for the examination of inflammatory response and is prepared from yeast, Saccharomyces cerevisiae. In the activation process, Toll-like receptor (TLR) 2 and TLR6 act as functional receptors for NF-kappaB activation. Although zymosan is primarily composed of beta-glucans, little is known about the active component of zymosan-mediated biological activities. The active moiety of zymosan was fractionated by its solubility in water, and its biological activity on macrophages and TLRs-transfectants examined. The macrophage cell line, RAW264.7, was treated with zymosan-derived preparations, and tumor necrosis factor alpha (TNF-alpha) produced in the culture supernatant was measured by ELISA. Increased TNF-alpha production was observed by stimulation with water-soluble (ZWS) or water-insoluble fraction (ZWIS). ZWS showed higher activity in TNF-alpha production. NF-kappaB activation via TLR2, TLR1/ TLR2, TLR2/TLR6, and TLR4/**MD-2**/CD14 also was enhanced by stimulation with ZWS and ZWIS. In particular, ZWS showed higher activity via TLR1/TLR2, TLR2/TLR6, and TLR4/**MD-2** /CD14 than other preparations. ZWS activity was decreased by treatment with polymyxin B, but not with lysozyme and zymolyase. Furthermore, ZWS contained significant more **endotoxin** than any other preparations. Therefore, we suggest that the active moiety of ZWS for the NF-kappaB activation has an **endotoxin**-like substance, that is abundantly observed in **Gram-negative** bacteria. These results imply that the inflammatory

activity of zymosan is induced not only by beta-glucans, but also by other **endotoxin**-like water-soluble substances.

L8 ANSWER 9 OF 30 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2005303700 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15949139
TITLE: Monomeric endotoxin:protein complexes are essential for TLR4-dependent cell activation.
AUTHOR: Gioannini T L; Teghanemt A; Zhang DeS; Levis E N; Weiss J P
CORPORATE SOURCE: Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa 52241, USA.. theresa-gioannini@uiowa.edu
CONTRACT NUMBER: AI59372 (NIAID)
P01AI44642 (NIAID)
SOURCE: Journal of endotoxin research, (2005) 11 (2) 117-23. Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 20050614
Last Updated on STN: 20050803
Entered Medline: 20050802

AB Potent TLR4-dependent cell activation by **Gram-negative** bacterial **endotoxin** depends on sequential **endotoxin**?protein and protein?protein interactions with LBP, CD14, **MD-2** and TLR4. LBP and CD14 combine, in an albumin-dependent fashion, to extract single **endotoxin** molecules from purified **endotoxin** aggregates (E(agg)) or the bacterial outer membrane and form monomeric **endotoxin**:CD14 complexes that are the preferred presentation of **endotoxin** for transfer to **MD-2**. **Endotoxin** in **endotoxin**:CD14is readily transferred to **MD-2**, again in an albumin-dependent manner, to form monomeric **endotoxin**:**MD-2** complex. This monomeric **endotoxin**:protein complex (**endotoxin**:**MD-2**) activates TLR4 at picomolar concentrations, independently of albumin, and is, therefore, the apparent ligand in **endotoxin**-dependent TLR4 activation. Tetra-, penta-, and hexa-acylated forms of **meningococcal endotoxin** (LOS) react similarly with LBP, CD14, and **MD-2** to form **endotoxin**:**MD-2** complexes. However, tetra- and penta-acylated LOS:**MD-2** complexes are less potent TLR4 agonists than hexa-acylated LOS:**MD-2**. This is mirrored in the reduced activity of tetra-, penta- versus hexa-acylated LOS aggregates (LOS(agg)) + LBP toward cells containing mCD14, **MD-2**, and TLR4. Therefore, changes in agonist potency of under-acylated meningococcal LOS are determined by differences in properties of monomeric **endotoxin**:**MD-2**.

L8 ANSWER 10 OF 30 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 2005303694 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15949133
TITLE: Detoxifying endotoxin: time, place and person.
AUTHOR: Munford Robert S
CORPORATE SOURCE: Molecular Host Defense Laboratory, Departments of

Internal Medicine and Microbiology, University of Texas Southwestern Medical School, Dallas, Texas 75390, USA.. robert.munford@utsouthwestern.edu
CONTRACT NUMBER: AI8188 (NIAID)
SOURCE: Journal of endotoxin research, (2005) 11 (2) 69-84.
Ref: 166
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 20050614
Last Updated on STN: 20050803
Entered Medline: 20050802

AB Animals that cannot sense **endotoxin** may die if they are infected by **Gram-negative** bacteria. Animals that sense **endotoxin** and respond too vigorously may also die, victims of their own inflammatory reactions. The outcome of **Gram-negative** bacterial infection is thus determined not only by an individual's ability to sense **endotoxin** and respond to its presence, but also by numerous phenomena that inactivate **endotoxin** and/or prevent harmful reactions to it. **Endotoxin** sensing requires the **MD-2**/TLR4 recognition complex and occurs principally in local tissues and the liver. This review highlights the known detoxification mechanisms, which include: (i) proteins that facilitate LPS sequestration by plasma lipoproteins, prevent interactions between the bioactive lipid A moiety and **MD-2**/TLR4, or promote cellular uptake via non-signaling pathway(s); (ii) enzymes that deacylate or dephosphorylate lipid A; (iii) mechanisms that remove LPS and **Gram-negative** bacteria from the bloodstream; and (iv) neuroendocrine adaptations that modulate LPS-induced mediator production or neutralize pro-inflammatory molecules in the circulation. In general, the mechanisms for sensing and detoxifying **endotoxin** seem to be compartmentalized (local versus systemic), dynamic, and variable between individuals. They may have evolved to confine infection and inflammation to extravascular sites of infection while preventing harmful systemic reactions. Integration of **endotoxin** sensing and detoxification is essential for successful host defense.

L8 ANSWER 11 OF 30 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 2004149149 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15010525
TITLE: Isolation of an endotoxin-MD-2 complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations.
AUTHOR: Gioannini Theresa L; Teghanemt Athmane; Zhang DeSheng; Coussens Nathan P; Dockstader Wendie; Ramaswamy S; Weiss Jerrold P
CORPORATE SOURCE: Inflammation Program, Department of Internal Medicine, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA.. theresa.gioannini@uiowa.edu
CONTRACT NUMBER: P01 44642
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2004 Mar 23) 101 (12)

4186-91. Electronic Publication: 2004-03-09.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 20040326
Last Updated on STN: 20040511
Entered Medline: 20040510

AB Host proinflammatory responses to minute amounts of **endotoxins** derived from many **Gram-negative** bacteria require the interaction of lipopolysaccharide-binding protein (LBP), CD14, Toll-like receptor 4 (TLR4) and **MD-2**. Optimal sensitivity to **endotoxin** requires an ordered series of **endotoxin**-protein and protein-protein interactions. At substoichiometric concentrations, LBP facilitates delivery of **endotoxin** aggregates to soluble CD14 (sCD14) to form monomeric **endotoxin**-sCD14 complexes. Subsequent interactions of **endotoxin**-sCD14 with TLR4 and/or **MD-2** have not been specifically defined. This study reports the purification of a stable, monomeric, bioactive **endotoxin-MD-2** complex generated by treatment of **endotoxin**-sCD14 with recombinant **MD-2**. Efficient generation of this complex occurred at picomolar concentrations of **endotoxin** and nanogram per milliliter doses of **MD-2** and required presentation of **endotoxin** to **MD-2** as a monomeric **endotoxin**-CD14 complex. TLR4-dependent delivery of **endotoxin** to human embryonic kidney (HEK) cells and cell activation at picomolar concentrations of **endotoxin** occurred with the purified **endotoxin-MD-2** complex, but not with purified **endotoxin** aggregates with or without LBP and/or sCD14. The presence of excess **MD-2** inhibited delivery of **endotoxin-MD-2** to HEK/TLR4 cells and cell activation. These findings demonstrate that TLR4-dependent activation of host cells by picomolar concentrations of **endotoxin** occurs by sequential interaction and transfer of **endotoxin** to LBP, CD14, and **MD-2** and simultaneous engagement of **endotoxin** and TLR4 by **MD-2**.

L8 ANSWER 12 OF 30 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 2004602663 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15472123
TITLE: Potential role of endotoxin as a proinflammatory mediator of atherosclerosis.
COMMENT: Comment in: Arterioscler Thromb Vasc Biol. 2005 May;25(5):e38; author reply e38-9. PubMed ID: 15863713
AUTHOR: Stoll Lynn L; Denning Gerene M; Weintraub Neal L
CORPORATE SOURCE: Department of Internal Medicine, Division of Cardiovascular Diseases, University of Iowa, Iowa City and The VA Medical Center, IA 52242, USA.. stolll@mail.medicine.uiowa.edu
SOURCE: Arteriosclerosis, thrombosis, and vascular biology, (2004 Dec) 24 (12) 2227-36. Electronic Publication: 2004-10-07. Ref: 175
Journal code: 9505803. ISSN: 1524-4636.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200506
ENTRY DATE: Entered STN: 20041204
Last Updated on STN: 20050622
Entered Medline: 20050621

AB Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Although a variety of inflammatory markers (ie, C-reactive protein) have been associated with atherosclerosis and its consequences, it is important to identify principal mediators of the inflammatory responses. One potentially important source of vascular inflammation in atherosclerosis is bacterial **endotoxin**. Mutations in Toll-like receptor 4 (TLR-4), an integral component of the **endotoxin** signaling complex, are fairly common in the Caucasian population and have recently been associated with reduced incidence of atherosclerosis and other cardiovascular diseases in some studies. Moreover, epidemiological studies suggest that endotoxemia at levels as low as 50 pg/mL constitutes a strong risk factor for the development of atherosclerosis. **Endotoxin** concentrations in this range may be produced by a variety of common subclinical **Gram-negative** infections. In this article, we outline the main elements of the **endotoxin** signaling receptor complex that initiates proinflammatory signaling (lipopolysaccharide binding protein [LBP], CD14, TLR-4, and **MD-2**) and discuss how changes in expression of these molecules may affect proatherogenic responses in the vessel wall. We also describe some of the proinflammatory effects of **endotoxin** that may be relevant to atherosclerosis, and discuss how serum lipoproteins, especially high-density lipoprotein, may modulate **endotoxin**-induced inflammatory responses. Further, we discuss recent findings suggesting that the lipid-lowering statins may have an additional protective role in blocking at least some of these proinflammatory signaling pathways. Finally, we discuss species diversity with regard to **endotoxin** signaling that should be considered when extrapolating experimental data from animal models to humans.

L8 ANSWER 13 OF 30 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 2004467314 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15339879
TITLE: Interaction of endotoxins with Toll-like receptor 4 correlates with their endotoxic potential and may explain the proinflammatory effect of Brucella spp. LPS.
AUTHOR: Duenas Ana I; Orduna Antonio; Crespo Mariano Sanchez; Garcia-Rodriguez Carmen
CORPORATE SOURCE: Unidad de Investigacion, Hospital Clinico Universitario, Valladolid, Spain.
SOURCE: International immunology, (2004 Oct) 16 (10) 1467-75. Electronic Publication: 2004-08-31. Journal code: 8916182. ISSN: 0953-8178.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200502
ENTRY DATE: Entered STN: 20040921

Searcher : Shears 571-272-2528

Last Updated on STN: 20050223
Entered Medline: 20050222

AB **Endotoxins** displaying differences in the chemical structure of their lipid A were used to induce the expression of chemokines in the human monocytic THP-1 cell line. LPS from two enterobacterial species such as Escherichia **coli** and Yersinia enterocolitica induced mRNA expression of IFN-gamma-inducible protein (IP)-10, macrophage-inflammatory protein (MIP)-1alpha, MIP-1beta, monocyte chemoattractant protein (MCP)-1 and IL-8. LPS from the non-enterobacterial genera Brucella and Ochrobactrum induced the expression of these chemokines to a lower extent. Attempts to address the signaling routes involved in these responses were carried out in transiently transfected HEK293 cells. Induction of kappaB-driven transcriptional activity by enterobacterial LPS was observed in cells transfected with TLR-4 alone, although co-transfection of TLR-4, **MD-2** and CD14 provided optimal induction. The response to Brucella spp. and Ochrobactrum anthropi LPS was only significant at the concentration of 10 microg/ml. These data indicate that LPS from Brucella spp. and O. anthropi, which contain lipid A moieties with structural features different from those of Enterobacteriaceae elicit biochemical signaling via TLR-4 only at high concentrations. Neither TLR-1, TLR-2 and TLR-6 nor heterodimeric combinations of these receptor molecules are involved. Conversely, the ability of LPS to activate the TLR-4 route is a reliable molecular biomarker for endotoxicity.

L8 ANSWER 14 OF 30 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 2004342289 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15121639
TITLE: Endotoxin responsiveness of human airway epithelia is limited by low expression of MD-2.
AUTHOR: Jia Hong Peng; Kline Joel N; Penisten Andrea; Apicella Michael A; Gioannini Theresa L; Weiss Jerrold; McCray Paul B Jr
CORPORATE SOURCE: Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA.
CONTRACT NUMBER: AI-24616 (NIAID)
AI-44642 (NIAID)
AI-65298 (NIAID)
ES-005605 (NIEHS)
HL-59324 (NHLBI)
HL-62134 (NHLBI)
P30 DK-54759 (NIDDK)
SOURCE: American journal of physiology. Lung cellular and molecular physiology, (2004 Aug) 287 (2) L428-37. Electronic Publication: 2004-04-30. Journal code: 100901229. ISSN: 1040-0605.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 20040713
Last Updated on STN: 20040818
Entered Medline: 20040817

AB The expression of inducible antimicrobial peptides, such as human beta-defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the

Toll-like receptors (TLRs). We found that primary cultures of well-differentiated human airway epithelia express the mRNA for TLR-4, but little or no **MD-2** mRNA, and display little HBD-2 expression in response to treatment with purified **endotoxin** +/- LPS binding protein (LBP) and soluble CD14. Expression of endogenous **MD-2** by transduction of airway epithelial cells with an adenoviral vector encoding **MD-2** or extracellular addition of recombinant **MD-2** both increased the responses of airway epithelia to **endotoxin** + LBP and sCD14 by >100-fold, as measured by NF-kappaB-luciferase activity and HBD-2 mRNA expression. **MD-2** mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed Haemophilus **influenzae**, the P6 outer membrane protein from H. **influenzae**, or TNF-alpha + IFN-gamma). These findings suggest that **MD-2**, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to **endotoxin**. The regulation of **MD-2** expression in airway epithelia and pulmonary macrophages may serve as a means to modify **endotoxin** responsiveness in the airway.

L8 ANSWER 15 OF 30 MEDLINE on STN DUPLICATE 14

ACCESSION NUMBER: 2004000596 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14688118
TITLE: Neisseria meningitidis lipooligosaccharide structure-dependent activation of the macrophage CD14/Toll-like receptor 4 pathway.
AUTHOR: Zughaier Susu M; Tzeng Yih-Ling; Zimmer Shanta M; Datta Anup; Carlson Russell W; Stephens David S
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA.
CONTRACT NUMBER: 2 R01 AI033517-10 (NIAID)
SOURCE: Infection and immunity, (2004 Jan) 72 (1) 371-80. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200402
ENTRY DATE: Entered STN: 20040103
Last Updated on STN: 20040203
Entered Medline: 20040202

AB **Meningococcal** lipopoly(oligo)saccharide (LOS) is a major inflammatory mediator of fulminant **meningococcal** sepsis and meningitis. Highly purified wild-type **meningococcal** LOS and LOS from genetically defined mutants of Neisseria **meningitidis** that contained specific mutations in LOS biosynthesis pathways were used to confirm that **meningococcal** LOS activation of macrophages was CD14/Toll-like receptor 4 (TLR4)-**MD-2** dependent and to elucidate the LOS structural requirement for TLR4 activation. Expression of TLR4 but not TLR2 was required, and antibodies to both TLR4 and CD14 blocked **meningococcal** LOS activation of macrophages. **Meningococcal** LOS alpha or beta chain oligosaccharide structure did not influence CD14/TLR4-**MD-2** activation. However, **meningococcal** lipid A, expressed by **meningococci** with defects in 3-deoxy-D-manno-octulosonic acid (KDO) biosynthesis or transfer,

resulted in an approximately 10-fold ($P < 0.0001$) reduction in biologic activity compared to KDO2-containing **meningococcal** LOS. Removal of KDO2 from LOS by acid hydrolysis also dramatically attenuated cellular responses. Competitive inhibition assays showed similar binding of glycosylated and unglycosylated lipid A to CD14/TLR4-**MD-2**. A decrease in the number of lipid A phosphate head groups or penta-acylated **meningococcal** LOS modestly attenuated biologic activity. **Meningococcal endotoxin** is a potent agonist of the macrophage CD14/TLR4-**MD-2** receptor, helping explain the fulminant presentation of **meningococcal** sepsis and meningitis. KDO2 linked to **meningococcal** lipid A was structurally required for maximal activation of the human macrophage TLR4 pathway and indicates an important role for KDO-lipid A in **endotoxin** biologic activity.

L8 ANSWER 16 OF 30 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 2004475343 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15379597
TITLE: Endotoxin recognition molecules MD-2 and toll-like receptor 4 as potential targets for therapeutic intervention of endotoxin shock.
AUTHOR: Miyake Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, 108-8639, Japan.. kmiyake@ims.u-tokyo.ac.jp
SOURCE: Current drug targets. Inflammation and allergy, (2004 Sep) 3 (3) 291-7. Ref: 88
Journal code: 101160019. ISSN: 1568-010X.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200412
ENTRY DATE: Entered STN: 20040925
Last Updated on STN: 20041229
Entered Medline: 20041228

AB **Gram-negative** sepsis is the major cause of deaths in intensive care units of hospitals and continues to increase worldwide due to the increased frequency of invasive procedures and therapy leading to immunosuppression. This syndrome is characterized by endothelial damage, coagulopathy, loss of vascular tone, tissue hypoperfusion, and multiple-organ failure. They are caused by uncontrolled, overwhelming inflammatory responses, which are triggered by microbial products. Amongst these products, **endotoxin** also called LPS (lipopolysaccharide), a constituent of the outer membrane of **Gram-negative** bacteria, is known to play a central role by eliciting immune responses leading to production of proinflammatory cytokines. Our understanding of LPS recognition has increased dramatically over the last several years by identification of Toll-like receptor 4 (TLR4) and **MD-2** as LPS recognition molecules. TLR4 is a mammalian homologue of drosophila Toll. The extracellular domain of TLR4 is associated with a molecule called **MD-2**. Mice lacking either TLR4 or **MD-2** do not respond to LPS and are resistant to **endotoxin** shock. Here, the potential for TLR4-

MD-2 as target molecules for therapeutic intervention is discussed.

L8 ANSWER 17 OF 30 JICST-EPlus COPYRIGHT 2006 JST on STN
ACCESSION NUMBER: 1050343423 JICST-EPlus
TITLE: Research on the analysis of biofunctions for drug discovery. Analysis of the mechanism of biofunctions mediated by lipid membrane domain like raft in animal cells and its relation to diseases.
AUTHOR: KITAGAWA TAKAYUKI; NISHIJIMA MASAHIRO
KUMAZAWA YOSHIO
TANAKA SHIGENORI
NAMBA KENJI
CORPORATE SOURCE: National Inst. Infectious Diseases, JPN
Kitasato Univ., Sch. of Sci.
Seikagakukogyo Chuken
Daiichiseiyaku Soyakuichiken
SOURCE: Soyakuto Hyuman Saiensu Kenkyu Sogo Kenkyu Hokokusho Heisei 13-15 Nendo Dai2 Bun'ya Soyaku no tameno Seitai Kino Kaiseki ni kansuru Kenkyu, (2004) pp. 114-118. Journal Code: N20050926 (Ref. 9)
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Short Communication
LANGUAGE: Japanese
STATUS: New
AB The distribution pattern of membrane transport proteins associated with lipid membrane domain like raft in animal cells was clarified. In a study to elucidate the mechanism of TLR4-**Md-2** complex to recognize foreign matter, new ligands were searched for and amino acid-containing membrane lipids of such **gram-negative** pathogens as taxol, Boredetella pertussis, Pseudomonas **aeruginosa**, and the like were found to activate NFKB through TLR4- **MD-2** complex on macrophage cell surface as LRS does. In addition, a model mouse system was used to analyze relationships of arteriosclerosis to **endotoxin** shock and pneumonic chlamydia infection and to study applications of the findings to preventive and therapeutic drugs for the infection.

L8 ANSWER 18 OF 30 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN
ACCESSION NUMBER: 2004308591 EMBASE
TITLE: Endotoxin responsiveness of human airway epithelia is limited by low expression of MD-2.
AUTHOR: Jia H.P.; Kline J.N.; Penisten A.; Apicella M.A.; Gioannini T.L.; Weiss J.; McCray Jr. P.B.
CORPORATE SOURCE: P.B. McCray Jr., Dept. of Pediatrics, Carver College of Medicine, Univ. of Iowa, Iowa City, IA 52242, United States. paul-mccray@uiowa.edu
SOURCE: American Journal of Physiology - Lung Cellular and Molecular Physiology, (2004) Vol. 287, No. 2 31-2, pp. L428-L437.
Refs: 58
ISSN: 1040-0605 CODEN: APLPE7
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 015 Chest Diseases, Thoracic Surgery and Tuberculosis
026 Immunology, Serology and Transplantation
LANGUAGE: English

Searcher : Shears 571-272-2528

SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040812
Last Updated on STN: 20040812

AB The expression of inducible antimicrobial peptides, such as human β-defensin-2 (HBD-2) by epithelia, comprises a component of innate pulmonary defenses. We hypothesized that HBD-2 induction in airway epithelia is linked to pattern recognition receptors such as the Toll-like receptors (TLRs). We found that primary cultures of well-differentiated human airway epithelia express the mRNA for TLR-4, but little or no **MD-2** mRNA, and display little HBD-2 expression in response to treatment with purified **endotoxin** ± LPS binding protein (LBP) and soluble CD14. Expression of endogenous **MD-2** by transduction of airway epithelial cells with an adenoviral vector encoding **MD-2** or extracellular addition of recombinant **MD-2** both increased the responses of airway epithelia to **endotoxin** + LBP and sCD14 by > 100-fold, as measured by NF-KB-luciferase activity and HBD-2 mRNA expression. **MD-2** mRNA could be induced in airway epithelia by exposure of these cells to specific bacterial or host products (e.g., killed Haemophilus **influenzae**, the P6 outer membrane protein from H. **influenzae**, or TNF-α + IFN-γ). These findings suggest that **MD-2**, either coexpressed with TLR-4 or secreted when produced in excess of TLR-4 from neighboring cells, is required for airway epithelia to respond sensitively to **endotoxin**. The regulation of **MD-2** expression in airway epithelia and pulmonary macrophages may serve as a means to modify **endotoxin** responsiveness in the airway.

L8 ANSWER 19 OF 30 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER: 2004-027278 [03] WPIDS
DOC. NO. NON-CPI: N2004-021623
DOC. NO. CPI: C2004-009400
TITLE: Transgenic non human animal with no response property to Gram negative bacterial membrane component e.g., lipopolysaccharide, comprises MD-2 gene deficient chromosome which encodes toll-like receptor.
DERWENT CLASS: B04 D16 P14 S03
PATENT ASSIGNEE(S): (KAGA-N) KAGAKU GIJUTSU SHINKO JIGYODAN
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 2003319734	A	20031111	(200403)*		13

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 2003319734	A	JP 2002-130964	20020502

PRIORITY APPLN. INFO: JP 2002-130964 20020502
AN 2004-027278 [03] WPIDS
AB JP2003319734 A UPAB: 20040112
NOVELTY - Transgenic non-human animal (I) with no response property to **Gram negative** bacterial membrane component e.g.,

lipopolysaccharide (LPS), comprises **MD-2** gene deficient chromosome which encodes toll-like receptor 4 (TLR4).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) screening (M1) a of **Gram-negative** -bacterial membrane component responsive substance, involves introducing a test substance into (I), or introducing a test substance into (I) having **MD-2** gene of different animal; and

(2) diagnosing (M2) the response of different **MD-2** gene in non-human animal, involves transducing **MD-2** gene into (I) and inducing an **endotoxin** shock into (I).

USE - (I) is useful for screening of **Gram-negative**-bacterial membrane component responsive substance, or for diagnosing the response of different **MD-2** genes in non-human animal (claimed). (I) is useful for developing a medical agent which is used for further drug development.

ADVANTAGE - (I) enables to screen **Gram-negative** -bacterial membrane component responsive substance, or to diagnose the response of different **MD-2** gene in non-human animal.

DESCRIPTION OF DRAWING(S) - The figure shows the lipopolysaccharide expression of the macrophage or dendritic cells derived from the **MD-2** genetically engineered mouse. (Drawing includes non-English language text).

Dwg.3/5

L8 ANSWER 20 OF 30 MEDLINE on STN DUPLICATE 16

ACCESSION NUMBER: 2003148258 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12604686
TITLE: Inhibition of endotoxin response by e5564, a novel Toll-like receptor 4-directed endotoxin antagonist.
AUTHOR: Mullarkey Maureen; Rose Jeffrey R; Bristol John; Kawata Tsutomu; Kimura Akufumi; Kobayashi Seiichi; Przetak Melinda; Chow Jesse; Gusovsky Fabian; Christ William J; Rossignol Daniel P
CORPORATE SOURCE: Biology Section, Eisai Research Institute of Boston, Inc., Andover, Massachusetts, USA.
SOURCE: Journal of pharmacology and experimental therapeutics, (2003 Mar) 304 (3) 1093-102.
Journal code: 0376362. ISSN: 0022-3565.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 20030401
Last Updated on STN: 20030422
Entered Medline: 20030421

AB Alpha-D-glucopyranose,3-O-decyl-2-deoxy-6-O-[2-deoxy-3-O-[(3R)-3-methoxydecyl]-6-O-methyl-2-[[(11Z)-1-oxo-11-octadecenyl]amino]-4-O-phosphono-beta-D-glucopyranosyl]-2-[(1,3-dioxotetradecyl)amino]-1-(dihydrogen phosphate), tetrasodium salt (E5564) is a second-generation synthetic lipodisaccharide designed to antagonize the toxic effects of **endotoxin**, a major immunostimulatory component of the outer cell membrane of **Gram negative** bacteria. In vitro, E5564 dose dependently (nanomolar concentrations) inhibited lipopolysaccharide (LPS)-mediated activation of primary cultures of human myeloid cells and mouse tissue

culture macrophage cell lines as well as human or animal whole blood as measured by production of tumor necrosis factor-alpha and other cytokines. E5564 also blocked the ability of **Gram negative** bacteria to stimulate human cytokine production in whole blood. In vivo, E5564 blocked induction of LPS-induced cytokines and LPS or bacterial-induced lethality in primed mice. E5564 was devoid of agonistic activity when tested both in vitro and in vivo and has no antagonistic activity against Gram positive-mediated cellular activation at concentrations up to 1 microM. E5564 blocked LPS-mediated activation of nuclear factor-kappaB in toll-like receptor 4/**MD-2** -transfected cells. In a mouse macrophage cell line, activity of E5564 was independent of serum, suggesting that E5564 exerts its activity through the cell surface receptor(s) for LPS, without the need for serum LPS transfer proteins. Similar to (6-O-[2-deoxy-6-O-methyl-4-O-phosphono-3-O-[(R)-3-Z-dodec-5-endoyloxydecl]-2-[3-oxo-tetradecanoylamino]-beta-O-phosphono-alpha-D-glucopyranose tetrasodium salt (E5531), another lipid A-like antagonist, E5564 associates with plasma lipoproteins, causing low concentrations of E5564 to be quantitatively inactivated in a dose- and time-dependent manner. However, compared with E5531, E5564 is a more potent inhibitor of cytokine generation, and higher doses retain activity for durations likely sufficient to permit clinical application. These results indicate that E5564 is a potent antagonist of LPS and lacks agonistic activity in human and animal model systems, making it a potentially effective therapeutic agent for treatment of disease states caused by **endotoxin**.

L8 ANSWER 21 OF 30 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 2003468338 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14517279
TITLE: Lipopolysaccharide interaction with cell surface Toll-like receptor 4-MD-2: higher affinity than that with MD-2 or CD14.
AUTHOR: Akashi Sachiko; Saitoh Shin-ichiroh; Wakabayashi Yasutaka; Kikuchi Takane; Takamura Noriaki; Nagai Yoshinori; Kusumoto Yutaka; Fukase Koichi; Kusumoto Shoichi; Adachi Yoshiyuki; Kosugi Atsushi; Miyake Kensuke
CORPORATE SOURCE: Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, Tokyo 108-8639, Japan.
SOURCE: Journal of experimental medicine, (2003 Oct 6) 198 (7) 1035-42. Electronic Publication: 2003-09-29. Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 20031008
Last Updated on STN: 20031113
Entered Medline: 20031112
AB Toll-like receptors (TLRs) are innate recognition molecules for microbial products, but their direct interactions with corresponding ligands remain unclarified. LPS, a membrane constituent of **gram-negative** bacteria, is the best-studied TLR ligand and is recognized by TLR4 and **MD-2**, a molecule associated with the extracellular domain of TLR4. Although

TLR4-**MD-2** recognizes LPS, little is known about the physical interaction between LPS and TLR4-**MD-2**. Here, we demonstrate cell surface LPS-TLR4-**MD-2** complexes. CD14 greatly enhances the formation of LPS-TLR4-**MD-2** complexes, but is not coprecipitated with LPS-TLR4-**MD-2** complexes, suggesting a role for CD14 in LPS loading onto TLR4-**MD-2** but not in the interaction itself between LPS and TLR4-**MD-2**. A tentative dissociation constant (Kd) for LPS-TLR4-**MD-2** complexes was approximately 3 nM, which is approximately 10-20 times lower than the reported Kd for LPS-**MD-2** or LPS-CD14. The presence of detergent disrupts LPS interaction with CD14 but not with TLR4-**MD-2**. E5531, a lipid A antagonist developed for therapeutic intervention of **endotoxin** shock, blocks LPS interaction with TLR4-**MD-2** at a concentration 100 times lower than that required for blocking LPS interaction with CD14. These results reveal direct LPS interaction with cell surface TLR4-**MD-2** that is distinct from that with **MD-2** or CD14.

L8 ANSWER 22 OF 30 MEDLINE on STN DUPLICATE 18

ACCESSION NUMBER: 2004038098 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14733729
TITLE: Regulation of interactions of endotoxin with host cells.
AUTHOR: Gioannini Theresa L; Teghanemt Athmane; Zarember Kol A; Weiss Jerrold P
CORPORATE SOURCE: Department of Internal Medicine, Division of Infectious Diseases and The Inflammation Program, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242, USA.
CONTRACT NUMBER: DK 05472 (NIDDK)
P01 44642
SOURCE: Journal of endotoxin research, (2003) 9 (6) 401-8.
Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200408
ENTRY DATE: Entered STN: 20040124
Last Updated on STN: 20040817
Entered Medline: 20040816

AB Potent Toll-like receptor 4 (TLR4)-dependent cell activation by **endotoxin** requires lipopolysaccharide-binding protein (LBP) and CD14-dependent delivery of **endotoxin** to cells containing **MD-2** and TLR4. We have used metabolically labeled [(14)C] **meningococcal** lipooligosaccharide (LOS), purified recombinant **endotoxin**-binding proteins, and cultured endothelial cells to better define protein:**endotoxin** intermediates key in cell activation in the absence of functional membrane (m) CD14. Protein:**endotoxin** complexes or aggregates (agg) were purified by gel sieving and characterized by immunocapture and bio-assays. Cell activation closely correlated with LBP, albumin and soluble (s) CD14-dependent conversion of **endotoxin** agg (M(r) > or = 20 x 10(6)) to monomeric (M(r) approximately 55 x 10(3)) **endotoxin**:sCD14 complexes. Ordered interaction of LBP (+ albumin) and sCD14 with LOSagg was required for the efficient formation of a bioactive **endotoxin**

:sCD14 complex and potent cell activation. Increasing the ratio of LBP/sCD14 or addition of bactericidal/permeability-increasing protein (BPI) reduced accumulation of **endotoxin**:sCD14 complexes and instead yielded aggregates of **endotoxin** (M(r) approximately 1-20 x 10(6)) containing LBP or BPI that were taken up by cells in a CD14- and TLR4-independent manner without inducing pro-inflammatory responses. These findings strongly suggest that host machinery linked to TLR4-dependent cellular activation or TLR4-independent cellular clearance of **endotoxin** selectively recognizes different protein:**endotoxin** complexes. At the outset of infection, the low concentrations of LBP present and absence of extracellular BPI favor formation of pro-inflammatory **endotoxin**:CD14 complexes. The mobilization of LBP and BPI that is triggered by inflammation directs **endotoxin** for clearance and hence resolution of **endotoxin**-triggered inflammation.

L8 ANSWER 23 OF 30 MEDLINE on STN DUPLICATE 19

ACCESSION NUMBER: 2002742036 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12505724
TITLE: Expression of recombinant proteins in a lipid A mutant of Escherichia coli BL21 with a strongly reduced capacity to induce dendritic cell activation and maturation.
AUTHOR: Cognet Isabelle; de Coignac Amelie Benoit; Magistrelli Giovanni; Jeannin Pascale; Aubry Jean-Pierre; Maisnier-Patin Karine; Caron Gersende; Chevalier Sylvie; Humbert Frederic; Nguyen Thien; Beck Alain; Velin Dominique; Delneste Yves; Malissard Martine; Gauchat Jean-Francois
CORPORATE SOURCE: Centre d'Immunologie Pierre-Fabre, 5 avenue Napoleon III, Saint-Julien en, Genevois, 74164, France.
SOURCE: Journal of immunological methods, (2003 Jan 15) 272 (1-2) 199-210.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021231
Last Updated on STN: 20030214
Entered Medline: 20030213

AB Mutations in the Escherichia **coli** (E. **coli**) and Salmonella lpxM gene have been shown to result in strains which grow normally and which produce a non-myristoylated lipopolysaccharide (nmLPS) with strongly reduced endotoxicity. Using homologous recombination, we inactivated the lpxM gene in BL21 (DE3), a strain widely used for the production of recombinant proteins. This led to a derivative unaffected in its capacity to support the production of recombinant proteins. This new strain expresses non-myristoylated LPS that induces markedly less activation and maturation of monocyte-derived dendritic cells (DC), as assessed by nuclear translocation of nuclear factor kappa B (NF-kappaB), production of TNF-alpha and IL-8 or expression of CD86. Activation of the main signal transducing receptor for extracellular LPS, Toll like receptor (TLR) 4 in conjunction with the soluble accessory protein **MD -2** was also markedly decreased.The modified BL21 strain represents a new application of lpxM inactivation for the expression of proteins to be tested on dendritic cells or other LPS sensitive

cells/receptor complexes. It is likely to be useful for the identification of new proteins activating the innate immune response and to reducing the risk linked with low level of **endotoxin** contamination in therapeutic recombinant proteins.

L8 ANSWER 24 OF 30 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:580809 BIOSIS
DOCUMENT NUMBER: PREV200300571406
TITLE: PROPIONIBACTERIUM ACNES TRIGGERS INFLAMMATORY RESPONSES VIA TOLL-LIKE RECEPTOR 2 AND SENSITIZES FOR LIVER INJURY VIA TLR2-INDEPENDENT PATHWAYS .
AUTHOR(S): Romics, Laszlo [Reprint Author]; Kodys, Karen [Reprint Author]; Golenbock, Douglas [Reprint Author]; Szabo, Gyongyi [Reprint Author]
CORPORATE SOURCE: Worcester, MA, USA
SOURCE: Digestive Disease Week Abstracts and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. S884. e-file. Meeting Info.: Digestive Disease 2003. FL, Orlando, USA. May 17-22, 2003. American Association for the Study of Liver Diseases; American Gastroenterological Association; American Society for Gastrointestinal Endoscopy; Society for Surgery of the Alimentary Tract.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Dec 2003
Last Updated on STN: 10 Dec 2003

AB Toll-like receptor 2 (TLR2), a pattern recognition receptor, recognizes gram-positive bacteria and lipoproteins. Propionibacterium acnes (P. acnes), a gram-positive bacterium, is a macrophage and Th1-cell activator that primes the liver to **endotoxin** induced injury modeling fulminant hepatitis. We and others recently showed that P. acnes mediates cell activation via TLR2. Thus, the aim of this study was to investigate the role of TLR2 in P. acnes induced priming of the liver to LPS-induced injury in vivo.METHODS: 6-8 week old C57BL/6 (WT; 3/group) or TLR2 deficient (-/-) mice were challenged with heat-killed P. acnes (1 mg, i.p.) or the TLR2 ligands, PGN and/or LTA (each 5ug/g b.w. i.p., Staph. A.), stimulated with LPS (0.5 mg/g b.w., E. **coli** 0111:B4) 7 days later and sacrificed at various timepoints. Serum TNFalpha, IL-12(p70), IL-6 and IFNgamma (ELISA), liver IL-12p40, IFNgamma, IL-1alpha, IL-1beta, IL-1Ra, IL-10 and IL-6 RNA (RNase protection assay) levels, and liver histopathology (HEPSILON) were assessed.RESULTS: P. acnes induced NF-kappaB activation in CHO cells expressing human TLR2/CD14 but not in CHO TLR4/CD14 cells. TLR2-mediated cell activation by P. acnes (10-100 mug/ml) was further suggested by up-regulation of IL-8 production ($p<.0004$) in TLR2, but not in TLR4/**MD-2** transfected HEK cells. However, investigation of the TLR2-mediated pathways revealed that P. acnes induced granuloma formation as well as sensitization for LPS-induced liver injury both in WT and in TLR2 -/- mice. P. acnes augmented LPS-induced serum TNFalpha, IL-6 and IFNgamma, but not IL-12 levels; liver cytokine RNA levels were increased both in WT and TLR2-/- mice. Finally, unlike P. acnes, selective TLR2 ligands (PGN and/or LTA) failed to sensitize for LPS induced injury evidenced by the lack of serum cytokine increase or liver granulomas.CONCLUSIONS: Our data demonstrate that P. acnes induces activation of inflammatory pathways via TLR2. However, selective activation via TLR2 is not sufficient to substitute for the

liver-sensitizing effects of P. acnes. The observation that liver sensitization by P. acnes occured in the absence of TLR2 expression suggest involvement of mechanism(s) other than TLR2-mediated pathways priming of the liver by P. acnes..

L8 ANSWER 25 OF 30 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 2002130979 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11859134
TITLE: Response to Neisseria gonorrhoeae by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling.
AUTHOR: Fichorova Raina N; Cronin Amanda O; Lien Egil; Anderson Deborah J; Ingalls Robin R
CORPORATE SOURCE: Fearing Research Laboratory, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA.
CONTRACT NUMBER: 5U19 AI 38515 (NIAID)
K08 AI 01476 (NIAID)
P01 AI 46518 (NIAID)
R01 AI 46613 (NIAID)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2002 Mar 1) 168 (5) 2424-32.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020228
Last Updated on STN: 20020317
Entered Medline: 20020315

AB Toll-like receptors (TLRs) have recently been identified as fundamental components of the innate immune response to bacterial pathogens. We investigated the role of TLR signaling in immune defense of the mucosal epithelial cells of the lower female genital tract. This site provides first line defense against microbial pathogens while remaining tolerant to a complex biosystem of resident microbiota. Epithelial cells derived from normal human vagina, ectocervix, and endocervix expressed mRNA for TLR1, -2, -3, -5, and -6. However, they failed to express TLR4 as well as **MD2**, two essential components of the receptor complex for LPS in phagocytes and endothelial cells. Consistent with this, endocervical epithelial cells were unresponsive to protein-free preparations of lipooligosaccharide from Neisseria gonorrhoeae and LPS from Escherichia **coli**. However, they were capable of responding to whole **Gram-negative** bacteria and bacterial lysates, as demonstrated by NF-kappaB activation and proinflammatory cytokine production. The presence of soluble CD14, a high-affinity receptor for LPS and other bacterial ligands, enhanced the sensitivity of genital tract epithelial cells to both low and high concentrations of bacteria, suggesting that soluble CD14 can act as a coreceptor for non-TLR4 ligands. These data demonstrate that the response to N. gonorrhoeae and other **Gram-negative** bacteria at the mucosal surface of the female genital tract occurs in the absence of **endotoxin** recognition and TLR4-mediated signaling.

L8 ANSWER 26 OF 30 TOXCENTER COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2002:157795 TOXCENTER

DOCUMENT NUMBER: CA13804037477E
TITLE: Innate recognition of endotoxin from gram-negative bacteria
AUTHOR(S): Miyake, Kensuke
CORPORATE SOURCE: Div. Infectious Genetics, Dep. Microbiology Immunology, Inst. Med. Sci., Univ. Tokyo, Japan.
SOURCE: Saishin Igaku, (2002) Vol. 57, No. 5, pp. 992-996. CODEN: SAIGAK. ISSN: 0370-8241.
COUNTRY: JAPAN
DOCUMENT TYPE: Journal
FILE SEGMENT: CAPLUS
OTHER SOURCE: CAPLUS 2002:515211
LANGUAGE: Japanese
ENTRY DATE: Entered STN: 20020716
Last Updated on STN: 20030120

AB A review discusses the role of Toll-like receptor 4 and **MD-2** mol. in the recognition of **endotoxin** such as lipopolysaccharide from **gram-neg.** bacteria.

L8 ANSWER 27 OF 30 MEDLINE on STN DUPLICATE 21
ACCESSION NUMBER: 2003179137 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12697088
TITLE: Essential role of MD-2 in B-cell responses to lipopolysaccharide and Toll-like receptor 4 distribution.
AUTHOR: Miyake Kensuke; Nagai Yoshinori; Akashi Sachiko; Nagafuku Masakazu; Ogata Masato; Kosugi Atsushi
CORPORATE SOURCE: Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, Japan.. kmiyake@ims.u-tokyo.ac.jp
SOURCE: Journal of endotoxin research, (2002) 8 (6) 449-52. Journal code: 9433350. ISSN: 0968-0519.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 20030417
Last Updated on STN: 20030725
Entered Medline: 20030724

AB Toll-like receptor 4 (TLR4) mediates lipopolysaccharide (LPS) signaling in a variety of cell types. **MD-2** is associated with the extracellular domain of TLR4 and augments TLR4-dependent LPS responses in vitro. Moreover, mice lacking **MD-2** (**MD-2**(-/-)) do not respond to LPS, survive **endotoxin** shock, and are susceptible to Salmonella **typhimurium** infection. Here, we further show that B cells lacking **MD-2** do not up-regulate CD23 in response to LPS. TLR4 predominantly resides in the Golgi apparatus without **MD-2**. **MD-2** is essential for LPS responses in vivo.

L8 ANSWER 28 OF 30 MEDLINE on STN DUPLICATE 22
ACCESSION NUMBER: 2001306548 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11257452
TITLE: LPS induction of gene expression in human monocytes.
AUTHOR: Guha M; Mackman N
CORPORATE SOURCE: Departments of Immunology, C-204, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA

92037, USA.
CONTRACT NUMBER: HL48872 (NHLBI)
SOURCE: Cellular signalling, (2001 Feb) 13 (2) 85-94. Ref: 152
Journal code: 8904683. ISSN: 0898-6568.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010604
Last Updated on STN: 20010604
Entered Medline: 20010531

AB Lipopolysaccharide (LPS [**endotoxin**]) is the principal component of the outer membrane of **Gram-negative** bacteria. Recent studies have elucidated how LPS is recognized by monocytes and macrophages of the innate immune system. Human monocytes are exquisitely sensitive to LPS and respond by expressing many inflammatory cytokines. LPS binds to LPS-binding protein (LBP) in plasma and is delivered to the cell surface receptor CD14. Next, LPS is transferred to the transmembrane signaling receptor toll-like receptor 4 (TLR4) and its accessory protein **MD2**. LPS stimulation of human monocytes activates several intracellular signaling pathways that include the IkappaB kinase (IKK)-NF-kappaB pathway and three mitogen-activated protein kinase (MAPK) pathways: extracellular signal-regulated kinases (ERK) 1 and 2, c-Jun N-terminal kinase (JNK) and p38. These signaling pathways in turn activate a variety of transcription factors that include NF-kappaB (p50/p65) and AP-1 (c-Fos/c-Jun), which coordinate the induction of many genes encoding inflammatory mediators.

L8 ANSWER 29 OF 30 JICST-EPlus COPYRIGHT 2006 JST on STN
ACCESSION NUMBER: 1020150200 JICST-EPlus
TITLE: Research on elucidation of mechanism of species specificity of the endotoxin action and application of medical supply to effectiveness and safety assessment (human science promotion foundation S).
AUTHOR: TANAMOTO KEN'ICHI; MUROI MASASHI
CORPORATE SOURCE: National Inst. Health Sci., JPN
SOURCE: Soyakuto Hyuman Saiensu Kenkyu Juten Kenkyu Hokokusho. Heisei 12 Nendo. Dai7 Bun'ya. Hito Soshiki o Mochiita Yakubutsu no Yukouseiu Anzensei ni kansuru Kenkyu, (2001) pp. 45-54. Journal Code: N20020048 (Fig. 11, Ref. 5)
PUB. COUNTRY: Japan
DOCUMENT TYPE: Journal; Short Communication
LANGUAGE: Japanese
STATUS: New

AB The problem of the species specificity of the **endotoxin** action is an important problem in development of the therapy of the **endotoxin** disease, biological functioning of the activity, medical supply evaluation by the pollution **endotoxin**. Being the inactivation in the human cell, salmonella lipid A showed the powerful activity in the mouse cell, and it was found that **MD -2** was a primary cause. And, it was found that the factor which activates the cell through TLR2 was included in LPS and lipid A of a Escherichia **coli** derivation.

L8 ANSWER 30 OF 30 TOXCENTER COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 2003:157319 TOXCENTER
DOCUMENT NUMBER: CRISP-2002-GM37696-160010
TITLE: 3D structure of LPS binding proteins and CD14
AUTHOR(S): TEYTON L
CORPORATE SOURCE: SCRIPPS RESEARCH INSTITUTE, 10550 NORTH TORREY PINES ROAD, LA JOLLA, CA 92037:CALIFORNIA
SUPPORTING ORGANIZATION (SPONSORING AGENCY): U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INSTITUTES OF HEALTH, NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES
SOURCE: Crisp Data Base National Institutes of Health.
DOCUMENT TYPE: (Research)
FILE SEGMENT: CRISP
LANGUAGE: English
ENTRY DATE: Entered STN: 20030708
Last Updated on STN: 20030708

AB GRANT=6576426;P01GM **Endotoxin** (lipopolysacharide or LPS) is the principal pro-inflammatory component of **Gram negative** bacteria.. Detection of LPS by the host innate immune system constitutes the first step in defense mechanisms against these pathogens. The amplitude of this response determines largely the outcome of these infections with the occurrence of a clinical septic shock in the pro-inflammatory burst is overwhelming. A number of proteins have been shown to bind LPS and be critical in LPS responses. However, the structural basis for binding, exchange, processing and association between those different proteins is largely unknown. To address this question we have initiated systematic studies that use recombinant forms of the different LPS binding molecules produced in a fly expression system. These recombinant molecules are used to carry out biological studies (LPS binding) and structural studies (x-ray crystallography). Initial studies have been centered around two long known LPS binding proteins: CD14 and LPB. Both have been expressed, purified to homogeneity and shown to bind LPS in an in vitro assay. Small crystals of LBP have been obtained but were too small to allow x-ray studies Large crystals of CD14 have been grown and used for x-ray diffraction experiments. Large crystals of CD14 have been grown and used for x-ray diffraction experiments. Native data sets have a medium resolution of approximately 3.1 Angstroms. To solve the phasing problem (in the absence of homologous known protein structure) we have expressed selenomethionein-derivitized CD14 in order to carry out MADD phasing experiments. Initial selenomethionein-containing crystals diffracted poorly. Improvement in crystallization has allowed diffraction to 3.8 Angstroms and we are currently trying to obtain phases from these data in order to do an initial map building. We were also able to characterize the natural lipids bounds to recombinant CD14 and LBP by mass spectrometry and would initiated single lipid loading experiments with CD14. This approach could lead to better resolution and would unveil the structural basis to lipid binding to CD14. This structure will direct our mutagenesis of CD14. A similar strategy is followed for other LPS-binding molecules, **MD2**, THR2, TLR4, NOD-1, NOD-2, in order to determine the general rules of LPS recognition by the innate immune system.

(FILE 'CAPLUS' ENTERED AT 11:07:07 ON 17 JAN 2006)
L1 2 SEA FILE=REGISTRY ABB=ON PLU=ON ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR)"/CN OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246)"/CN)

L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON MD2/CN
L3 3 SEA FILE=REGISTRY ABB=ON PLU=ON "MD 2"/CN
L4 6 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2 OR L3
L9 76 SEA FILE=CAPLUS ABB=ON PLU=ON (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?)(2W)2) AND (ENDOTOXIN OR ENDO TOXIN)
L10 12 SEA FILE=CAPLUS ABB=ON PLU=ON L9 AND (PURE OR PURIF?)
L11 11 SEA FILE=CAPLUS ABB=ON PLU=ON L10 AND (GRAM(W)(NEG OR NEGATIVE) OR MENINGITID? OR MENINGOCOCC? OR COLI OR AERUGINOSA OR INFLUENZAE OR TYPHIMURIUM OR TULARENSIS)

L12 1 L11 NOT L6

L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
ED Entered STN: 02 Sep 2003
ACCESSION NUMBER: 2003:682201 CAPLUS
DOCUMENT NUMBER: 139:306331
TITLE: Evidence of expression of **endotoxin** receptors CD14, Toll-like receptors TLR4 and TLR2 and associated molecule **MD-2** and of sensitivity to **endotoxin** (LPS) in islet beta cells
AUTHOR(S): Vives-Pi, M.; Somoza, N.; Fernandez-Alvarez, J.; Vargas, F.; Caro, P.; Alba, A.; Gomis, R.; Labeta, M. O.; Pujol-Borrell, R.
CORPORATE SOURCE: Laboratory of Immunobiology for Research and Diagnostic Applications, Transfusion Center and Tissue Bank, 'Germans Trias i Pujol' University Hospital, Badalona, Spain
SOURCE: Clinical and Experimental Immunology (2003), 133(2), 208-218
CODEN: CEXIAL; ISSN: 0009-9104
PUBLISHER: Blackwell Publishing Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CD14, a GPI-linked membrane protein, is a component of the lipopolysaccharide (LPS) receptor complex, one of the pattern-recognizing receptors (PRR) expressed by myeloid lineage cells. Here we report that CD14, the functionally linked toll-like receptor mols., TLR2 and TLR4, and the associated mol. **MD-2** are expressed in endocrine cells of the human pancreatic islets. CD14 expression in human pancreatic islets was determined by immunofluorescence staining of tissue sections and primary cultures, and confirmed by flow cytometry of dispersed normal islets and SV40-transformed islet cells (HP62). The latter cells synthesized and secreted CD14 in response to lipopolysaccharide (LPS) in a time- and dose-dependent manner. Reverse transcription polymerase chain reaction (RT-PCR)-Southern was pos. for CD14, TLR2, TLR4 and **MD-2** in human pancreas, **purified** islets and HP62 cells. In vitro expts. using rat islets (also pos. for CD14 by RT-PCR) and HP62 cells showed that LPS regulates glucose-dependent insulin secretion and induces inflammatory cytokines [interleukin (IL)-1α, IL-6 and tumor necrosis factor (TNF)-α]. The functional expression of CD14 and associated mols. in islet β cells adds a new pathway that islet cells may follow to adjust their function to endotoxemia situations and become vulnerable to the inflammatory events that occur during diabetogenic insulitis.
REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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     (FILE 'CAPLUS' ENTERED AT 11:00:34 ON 17 JAN 2006)
               DEL HIS Y

     FILE 'REGISTRY' ENTERED AT 11:01:28 ON 17 JAN 2006
               E "MD-2"/CN 7
L1             2 SEA ABB=ON  PLU=ON  ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR
                 )"/CN OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246
                 )"/CN)
               E MD2/CN 7
L2             1 SEA ABB=ON  PLU=ON  MD2/CN
               E MD 2/CN 7
L3             3 SEA ABB=ON  PLU=ON  "MD 2"/CN
               E MYELOID DIFFERENTIATION PROTEIN/CN
L4             6 SEA ABB=ON  PLU=ON  L1 OR L2 OR L3

     FILE 'CAPLUS' ENTERED AT 11:02:41 ON 17 JAN 2006
L5            59 SEA ABB=ON  PLU=ON  (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?
                 )(2W)2)(L)(ENDOTOXIN OR ENDO TOXIN)
L6            25 SEA ABB=ON  PLU=ON  L5(L)(GRAM(W)(NEG OR NEGATIVE) OR
                 MENINGITID? OR MENINGOCOCC? OR COLI OR AERUGINOSA OR
                 INFLUENZAE OR TYPHIMURIUM OR TULARENSIS)

     FILE 'REGISTRY' ENTERED AT 11:04:52 ON 17 JAN 2006

     FILE 'CAPLUS' ENTERED AT 11:04:52 ON 17 JAN 2006
               D QUE
               D 1-25 .BEVSTR

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,
     JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:04:55 ON 17 JAN 2006
L7           123 SEA ABB=ON  PLU=ON  L6
L8            30 DUP REM L7 (93 DUPLICATES REMOVED)
               D 1-30 IBIB ABS

     FILE 'HOME' ENTERED AT 11:06:42 ON 17 JAN 2006

     FILE 'CAPLUS' ENTERED AT 11:07:07 ON 17 JAN 2006
L9            76 SEA ABB=ON  PLU=ON  (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?
                 )(2W)2) AND (ENDOTOXIN OR ENDO TOXIN)
L10           12 SEA ABB=ON  PLU=ON  L9 AND (PURE OR PURIF?)
L11           11 SEA ABB=ON  PLU=ON  L10 AND (GRAM(W)(NEG OR NEGATIVE) OR
                 MENINGITID? OR MENINGOCOCC? OR COLI OR AERUGINOSA OR
                 INFLUENZAE OR TYPHIMURIUM OR TULARENSIS)
               D QUE
L12            1 SEA ABB=ON  PLU=ON  L11 NOT L6
               D .BEVSTR

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,
     JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:10:58 ON 17 JAN 2006
L13           55 SEA ABB=ON  PLU=ON  L11
L14            7 SEA ABB=ON  PLU=ON  L13 NOT L7
L15            5 DUP REM L14 (2 DUPLICATES REMOVED)
               D 1-5 IBIB ABS

     FILE 'CAPLUS' ENTERED AT 11:18:39 ON 17 JAN 2006
L16           10 SEA ABB=ON  PLU=ON  L6(L)(NEISSER? OR ESCHERICH? OR
                 PSEUDOMONAS OR HEMOPHILUS OR HAEMOPHILUS OR SALMONELLA OR
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                FRANCISELLA)
L17           6 SEA ABB=ON  PLU=ON  L10 AND (NEISSER? OR ESCHERICH? OR
                PSEUDOMONAS OR HEMOPHILUS OR HAEMOPHILUS OR SALMONELLA OR
                FRANCISELLA)
L18           0 SEA ABB=ON  PLU=ON  (L16 OR L17) NOT (L6 OR L12)
                D QUE L16
                D QUE L17

     FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,
     JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:20:43 ON 17 JAN 2006
L19          47 SEA ABB=ON  PLU=ON  L16
L20          31 SEA ABB=ON  PLU=ON  L17
L21           0 SEA ABB=ON  PLU=ON  (L19 OR L20) NOT (L7 OR L14)

     FILE 'HOME' ENTERED AT 11:22:07 ON 17 JAN 2006


     FILE REGISTRY
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     DICTIONARY FILE UPDATES:  15 JAN 2006  HIGHEST RN 871978-73-3

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     * available and contains the CA role and document type information. *
     *                                                          *
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FILE LAST UPDATED: 16 Jan 2006 (20060116/ED)

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FILE MEDLINE
FILE LAST UPDATED: 14 JAN 2006 (20060114/UP). FILE COVERS 1950 TO DA

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The MEDLINE reload for 2006 will soon be available. For details on the 2005 reload, enter HELP RLOAD at an arrow promt (=>).
See also:

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http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.ht
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

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This file contains CAS Registry Numbers for easy and accurate

FILE BIOSIS
FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 11 January 2006 (20060111/ED)

FILE EMBASE
FILE COVERS 1974 TO 12 Jan 2006 (20060112/ED)

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FILE WPIDS
FILE LAST UPDATED: 16 JAN 2006 <20060116/UP>
MOST RECENT DERWENT UPDATE: 200604 <200604/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

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http://www.stn-international.de/stndatabases/details/ipc_reform.html
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FILE CONFSCI
FILE COVERS 1973 TO 25 May 2005 (20050525/ED)

FILE SCISEARCH

FILE COVERS 1974 TO 11 Jan 2006 (20060111/ED)

SCISEARCH has been reloaded, see HELP RLOAD for details.

FILE JICST-EPLUS
FILE COVERS 1985 TO 10 JAN 2006 (20060110/ED)

THE JICST-EPLUS FILE HAS BEEN RELOADED TO REFLECT THE 1999 CONTROLLED
TERM (/CT) THESAURUS RELOAD.

FILE JAPIO
FILE LAST UPDATED: 02 JAN 2006 <20060102/UP>
FILE COVERS APR 1973 TO SEPTEMBER 29, 2005

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identification.

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TOXCENTER has been enhanced with new files segments and search fields.
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TOXCENTER thesauri in the /CN, /CT, and /MN fields incorporate the

MeSH 2006 vocabulary.
See http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.ht
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html
for a description of changes.

FILE HOME

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:10:58 ON 17 JAN 2006)
L13 55 S L11
L14 7 S L13 NOT L7
L15 5 DUP REM L14 (2 DUPLICATES REMOVED)

L15 ANSWER 1 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:1240080 SCISEARCH
THE GENUINE ARTICLE: 991MK
TITLE: Expression of a Porphyromonas gingivalis lipid A palmitylacyltransferase in Escherichia **coli** yields a chimeric lipid A with altered ability to stimulate interleukin-8 secretion
AUTHOR: Bainbridge B W; Coats S R; Pham T T T; Reife R A; Darveau R P (Reprint)
CORPORATE SOURCE: Univ Washington, Dept Oral Biol, Seattle, WA 98195 USA (Reprint); Univ Washington, Dept Periodont, Seattle, WA 98195 USA
rdarveau@u.washington.edu
COUNTRY OF AUTHOR: USA
SOURCE: CELLULAR MICROBIOLOGY, (JAN 2006) Vol. 8, No. 1, pp. 120-129.
ISSN: 1462-5814.
PUBLISHER: BLACKWELL PUBLISHING, 9600 GARSINGTON RD, OXFORD OX4 2DQ, OXON, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 49
ENTRY DATE: Entered STN: 22 Dec 2005
Last Updated on STN: 22 Dec 2005

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In Escherichia **coli** the gene htrB codes for an acyltransferase that catalyses the incorporation of laurate into lipopolysaccharide (LPS) as a lipid A substituent. We describe the cloning, expression and characterization of a Porphyromonas gingivalis htrB homologue. When the htrB homologue was expressed in wild-type E. **coli** or a mutant strain deficient in htrB, a chimeric LPS with altered lipid A structure was produced. Compared with wild-type E. **coli** lipid A, the new lipid A species contained a palmitate (C16) in the position normally occupied by laurate (C12) suggesting that the cloned gene performs the same function as E. **coli** htrB but preferentially transfers the longer-chain palmitic acid that is known to be present in P. gingivalis LPS. LPS was **purified** from wild-type E. **coli**, the E. **coli** htrB mutant strain and the htrB mutant strain expressing the P. gingivalis acyltransferase. LPS from the palmitate bearing chimeric LPS as well as the htrB mutant exhibited a reduced ability to activate human embryonic kidney 293 (HEK293) cells transfected with TLR4/**MD2**. LPS from the htrB mutant also had a greatly reduced ability to stimulate interleukin-8 (IL-8) secretion in both endothelial cells and monocytes. In contrast, the activity of LPS from the htrB mutant bacteria expressing the P. gingivalis gene displayed wild-type activity to stimulate IL-8 production from endothelial cells but a reduced ability to stimulate IL-8 secretion from monocytes. The intermediate activation observed in monocytes for the chimeric LPS was similar to the pattern seen in HEK293 cells expressing TLR4/**MD2** and CD14. Thus, the presence of a longer-chain fatty acid

on E. **coli** lipid A altered the activity of the LPS in monocytes but not endothelial cell assays and the difference in recognition does not appear to be related to differences in Toll-like receptor utilization.

L15 ANSWER 2 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:740824 SCISEARCH
THE GENUINE ARTICLE: 714FN
TITLE: Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells
AUTHOR: Smith M F (Reprint); Mitchell A; Li G L; Ding S; Fitzmaurice A M; Ryan K; Crowe S; Goldberg J B
CORPORATE SOURCE: Univ Virginia Hlth Syst, Dept Med, POB 800708, Charlottesville, VA 22908 USA (Reprint); Univ Virginia Hlth Syst, Dept Med, Charlottesville, VA 22908 USA; Univ Virginia Hlth Syst, Dept Digest Hlth Ctr Excellence, Charlottesville, VA 22908 USA; Univ Virginia Hlth Syst, Dept Microbiol, Charlottesville, VA 22908 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (29 AUG 2003) Vol. 278, No. 35, pp. 32552-32560.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34
ENTRY DATE: Entered STN: 12 Sep 2003
Last Updated on STN: 12 Sep 2003

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Infection with Helicobacter pylori, a **Gram-negative**, microaerophilic, flagellated bacteria that adheres to human gastric mucosa, is strongly associated with gastric ulcers and adenocarcinoma. The mechanisms through which gastric epithelial cells recognize this organism are unclear. In this study we evaluated the interactions between the Toll-like receptors (TLRs) and H. pylori-mediated NF-kappaB activation and the induction of chemokine mRNA expression. By reverse transcriptase-PCR we determined that MKN45 gastric epithelial cells express low but detectable amounts of TLR2, -4, and -5 but no **MD-2**. To determine which, if any, TLRs may play a role in the response of epithelial cells to H. pylori, HEK293 cells were cotransfected with the NF-kappaB-Luc reporter, CD14 and **MD2** expression plasmids, and expression plasmids for TLR2, TLR4, or TLR5. Infection of the cultures with H. pylori (strain 26695) induced NF-kappaB activity in cells transfected with TLR2 and TLR5, but not TLR4. Consistent with the HEK293 experiments, H. pylori-induced NF-kappaB activation was decreased in MKN45 gastric epithelial cells by transfection of dominant-negative versions of TLR2 and TLR5 but not TLR4. Highly **purified** lipopolysaccharide from H. pylori strain 26695 activated NF-kappaB in HEK293 via TLR2 but not TLR4. Partially **purified** flagellin from H. pylori was also capable of inducing NF-kappaB activation in HEK cells transfected with TLR5. Additionally, chemokine gene expression was induced by H. pylori in HEK293 cells following stable transfection with TLR2 or TLR5 expression plasmids. These studies

demonstrate that gastric epithelial cells recognize and respond to H. pylori infection at least in part via TLR2 and TLR5. Furthermore, the unique lipopolysaccharide of H. pylori is a TLR2, not a TLR4 agonist.

L15 ANSWER 3 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:831497 SCISEARCH
THE GENUINE ARTICLE: 481NU
TITLE: **MD-2** binds to bacterial lipopolysaccharide
AUTHOR: Viriyakosol S (Reprint); Tobias P S; Kitchens R L; Kirkland T N
CORPORATE SOURCE: Vet Adm San Diego Healthcare Syst, 9111F, 3350 La Jolla Village Dr, San Diego, CA 92161 USA (Reprint); Vet Adm San Diego Healthcare Syst, San Diego, CA 92161 USA; Univ Calif San Diego, Dept Pathol & Med, San Diego, CA 92161 USA; Scripps Res Inst, Dept Immunol, La Jolla, CA 92037 USA; Univ Texas, SW Med Ctr, Dept Internal Med, Dallas, TX 75390 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 OCT 2001) Vol. 276, No. 41, pp. 38044-38051.
ISSN: 0021-9258.
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 26
ENTRY DATE: Entered STN: 26 Oct 2001
Last Updated on STN: 26 Oct 2001

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The exact roles and abilities of the individual components of the lipopolysaccharide (LPS) receptor complex of proteins remain unclear. **MD-2** is a molecule found in association with toll-like receptor 4. We produced recombinant human **MD-2** to explore its LPS binding ability and role in the LPS receptor complex. **MD-2** binds to highly **purified** rough LPS derived from Salmonella minnesota and Escherichia **coli** in five different assays; one assay yielded an apparent KD of 65 nm. **MD-2** binding to LPS did not require LPS-binding proteins LBP and CD14; in fact LBP competed with **MD-2** for LPS : **MD-2** enhanced the biological activity of LPS in toll-like receptor 4 transfected Chinese hamster ovary cells but inhibited LPS activation of U373 astrocytoma cells and of monocytes in human whole blood. These data indicate that **MD-2** is a genuine LPS-binding protein and strongly suggest that **MD-2** could play a role in regulation of cellular activation by LPS, depending on its local availability.

L15 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:121588 SCISEARCH
THE GENUINE ARTICLE: 396EE
TITLE: **MD-2** enables toll-like receptor 2 (TLR2)-mediated responses to lipopolysaccharide and enhances TLR2-mediated responses to gram-positive and **gram-negative** bacteria and their cell wall components

Searcher : Shears 571-272-2528

AUTHOR: Dziarski R (Reprint); Wang Q L; Miyake K; Kirschning C J; Gupta D
CORPORATE SOURCE: Indiana Univ, Sch Med, NW Ctr Med Educ, 3400 Broadway, Gary, IN 46408 USA (Reprint); Indiana Univ, Sch Med, NW Ctr Med Educ, Gary, IN 46408 USA; Saga Med Sch, Dept Immunol, Saga, Japan; Tech Univ Munich, Inst Med Microbiol Immunol & Hyg, D-8000 Munich, Germany
COUNTRY OF AUTHOR: USA; Japan; Germany
SOURCE: JOURNAL OF IMMUNOLOGY, (1 FEB 2001) Vol. 166, No. 3, pp. 1938-1944.
ISSN: 0022-1767.
PUBLISHER: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29
ENTRY DATE: Entered STN: 18 Feb 2001
Last Updated on STN: 18 Feb 2001
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **MD-2** is associated with Toll-like receptor 4 (TLR4) on the cell surface and enables TLR4 to respond to LPS. We tested whether **MD-2** enhances or enables the responses of both TLR2 and TLR4 to **Gram-negative** and Gram-positive bacteria and their components. TLR2 without **MD-2** did not efficiently respond to highly **purified** LPS and LPS partial structures. **MD-2** enabled TLR2 to respond to nonactivating protein-free LPS, LPS mutants, or lipid A and enhanced TLR2-mediated responses to both **Gram-negative** and Gram-positive bacteria and their LPS, peptidoglycan, and lipoteichoic acid components. **MD-2** enabled TLR4 to respond to a wide variety of LPS partial structures, **Gram-negative** bacteria, and Gram-positive lipoteichoic acid, but not to Gram-positive bacteria, peptidoglycan, and lipopeptide, **MD-2** physically associated with TLR2, but this association was weaker than with TLR4, **MD-2** enhanced expression of both TLR2 and TLR4, and TLR2 and TLR4 enhanced expression of **MD-2**. Thus, **MD-2** enables both TLR4 and TLR2 to respond with high sensitivity to a broad range of LPS structures and to lipoteichoic acid, and, moreover, **MD-2** enhances the responses of TLR2 to Gram-positive bacteria and peptidoglycan, to which the TLR4-**MD-2** complex is unresponsive.

L15 ANSWER 5 OF 5 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 1
ACCESSION NUMBER: 2001:220894 BIOSIS
DOCUMENT NUMBER: PREV200100220894
TITLE: Role of **MD-2** in TLR2- and TLR4-mediated recognition of **Gram-negative** and Gram-positive bacteria and activation of chemokine genes.
AUTHOR(S): Dziarski, Roman [Reprint author]; Gupta, Dipika
CORPORATE SOURCE: Indiana University School of Medicine, 3400 Broadway, Gary, IN, 46408, USA
rdziar@iun.edu
SOURCE: Journal of Endotoxin Research, (2000) Vol. 6, No. 5, pp. 401-405. print.
ISSN: 0968-0519.
DOCUMENT TYPE: Article

Searcher : Shears 571-272-2528

LANGUAGE: English
ENTRY DATE: Entered STN: 9 May 2001
Last Updated on STN: 18 Feb 2002

AB **MD-2** is associated with TLR4 on the cell surface and enables TLR4 to respond to LPS. TLR2 without **MD-2** does not respond to **pure** protein-free endotoxic LPS, ReLPS, and lipid A. **MD-2** enables TLR2 to respond to non-activating LPS, ReLPS, and lipid A, and enhances TLR2-mediated responses to **Gram-negative** and Gram-positive bacteria, protein-containing LPS, peptidoglycan, and lipoteichoic acid. **MD-2** enables TLR4 to respond to a wide variety of endotoxic LPS partial structures, **Gram-negative** bacteria, and Gram-positive lipoteichoic acid, but not to Gram-positive bacteria, peptidoglycan, and lipopeptide. **MD-2** physically associates with both TLR4 and TLR2, but the association with TLR2 is weaker than with TLR4. Also, **MD-2** and TLR2 and TLR4 enhance each other's expression. The highest induced genes in human monocytes stimulated with Gram-positive and **Gram-negative** bacterial cell wall components are chemokine genes, and IL-8 is the highest induced chemokine. Both Gram-positive and **Gram-negative** bacteria activate TLR2fwdarwMyD88fwdarwIRAKfwdarwTRAF fwdarwNIKfwdarwIKKfwdarwNF-kappaB signal transduction pathway that induces transcription of the IL-8 gene. Therefore, TLR2 is a functional receptor for both Gram-positive and **Gram-negative** bacteria and it induces activation of IL-8.

FILE 'CAPLUS' ENTERED AT 11:18:39 ON 17 JAN 2006

L1 2 SEA FILE=REGISTRY ABB=ON PLU=ON ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR)"/CN OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246)"/CN)
L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON MD2/CN
L3 3 SEA FILE=REGISTRY ABB=ON PLU=ON "MD 2"/CN
L4 6 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2 OR L3
L5 59 SEA FILE=CAPLUS ABB=ON PLU=ON (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?)(2W)2)(L)(ENDOTOXIN OR ENDO TOXIN)
L6 25 SEA FILE=CAPLUS ABB=ON PLU=ON L5(L)(GRAM(W)(NEG OR NEGATIVE) OR MENINGITID? OR MENINGOCOCC? OR COLI OR AERUGINOSA OR INFLUENZAE OR TYPHIMURIUM OR TULARENSIS)
L16 10 SEA FILE=CAPLUS ABB=ON PLU=ON L6(L)(NEISSER? OR ESCHERICH? OR PSEUDOMONAS OR HEMOPHILUS OR HAEMOPHILUS OR SALMONELLA OR FRANCISELLA)

L1 2 SEA FILE=REGISTRY ABB=ON PLU=ON ("MD-2 PROTEIN (HUMAN CLONE 1 PRECURSOR)"/CN OR "MD-2 PROTEIN (HUMAN CLONE MGC:22424 IMAGE:4767246)"/CN)
L2 1 SEA FILE=REGISTRY ABB=ON PLU=ON MD2/CN
L3 3 SEA FILE=REGISTRY ABB=ON PLU=ON "MD 2"/CN
L4 6 SEA FILE=REGISTRY ABB=ON PLU=ON L1 OR L2 OR L3
L9 76 SEA FILE=CAPLUS ABB=ON PLU=ON (L4 OR MD2 OR (MD OR MYELOID DIFFERENT?)(2W)2) AND (ENDOTOXIN OR ENDO TOXIN)
L10 12 SEA FILE=CAPLUS ABB=ON PLU=ON L9 AND (PURE OR PURIF?)
L17 6 SEA FILE=CAPLUS ABB=ON PLU=ON L10 AND (NEISSER? OR ESCHERICH? OR PSEUDOMONAS OR HEMOPHILUS OR HAEMOPHILUS OR SALMONELLA OR FRANCISELLA)

L18 0 S (L16 OR L17) NOT (L6 OR L12)

Searcher : Shears 571-272-2528

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, TOXCENTER' ENTERED AT 11:20:43 ON 17 JAN 2006)

L19 47 S L16
L20 31 S L17
L21 0 S (L19 OR L20) NOT (L7 OR L14)

FILE 'HOME' ENTERED AT 11:22:07 ON 17 JAN 2006